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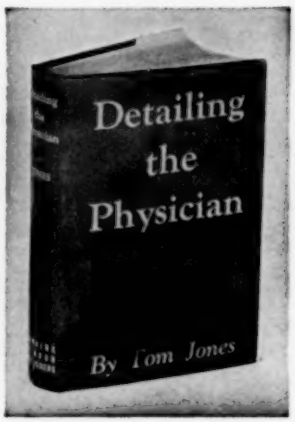

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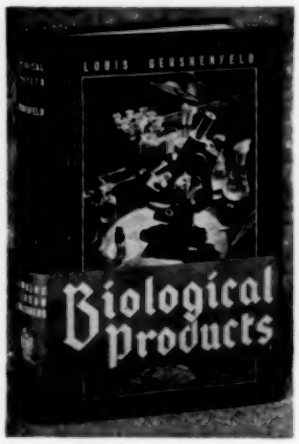

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VOLUME XXXI | *APRIL, 1942* | NUMBER 4 CONSECUTIVE No. 7

The Fungistatic Properties of Pyridine Carboxylic and Aminobenzoic Acids, a Resonance Effect*

By Charles Hoffman, T. R. Schweitzer and Gaston Dalby

The relationship of fungistatic properties to molecular structure is of considerable interest. The effect of many chemical substances on bacteria and mold varies with the *p*H of the media. Bacteria in general prefer the neutral *p*H ranges, but mold will grow readily from *p*H 2 to *p*H 8 thus affording a wide range of *p*H values for a study of fungistatic properties. The relationship of structure to mold-inhibiting power often is not apparent unless the effect of *p*H is considered.

Hoffman, *et al.* (1), studied the fungistatic properties of benzoic acid and related compounds. The introduction of a polar group into the benzene ring nucleus markedly affects the fungistatic properties of the molecule. If, for example, a hydroxyl group is introduced into the *ortho* position to form salicylic acid, the power of the molecule as a fungistat is decreased. If the hydroxyl is introduced into either the *meta* or *para* positions, the fungistatic properties are eliminated entirely. An analogous situation was shown to exist in the dihydroxybenzene compounds. Catechol is more powerfully fungistatic than resorcinol, and resorcinol more powerful than hydroquinone. Hoffman, *et al.* (1), therefore suggested the hypothesis that if the polar groups are concentrated in one area of the molecule, a more powerful fungistat results than if the polar groups are spaced around the non-polar nucleus.

The introduction of an amino group into the benzene ring nucleus of benzoic acid forms three isomeric aminobenzoic acids, one of which, *p*-aminobenzoic, has been recognized as a vitamin of the B-complex. The three isomeric pyridine carboxylic acids (including the important vitamin, nicotinic acid) have a skeleton structure similar to benzoic acid, with a nitrogen replacing a carbon in the ring. Since such a nitrogen is capable of forming a pyridonium ion, it acts as a polar group. These six compounds form, therefore, an interesting group of substances for the study of the relationship of structure to fungistatic properties.

Hoffman, *et al.* (2), reported a method for the determination of fungistatic properties. This method was used exactly as originally reported. The concentrations in the table and graph represent the minimum molar

* From the Ward Baking Company, New York, N. Y.

Table 1.—Molar Concentrations of Benzoic and Substituted Benzoic Acids Necessary to Inhibit Mold for 48 Hours at 37.5° C. at Various *p*H Values

	*p*H 2	*p*H 4	*p*H 5	*p*H 6	*p*H 7	*p*H 8
Benzoic acid	0.0016	..	0.0074	0.0532	(Ineffective above *p*H 6)	
Ortho (α) substitution						
Anthranilic acid	0.0058	..	0.0146	0.051	(Ineffective above *p*H 6)	
Picolinic acid	0.063	(Ineffective above *p*H 3.8)				
Meta (β) substitution						
m-Aminobenzoic acid	0.161	(Ineffective above *p*H 3)				
Nicotinic acid	(Ineffective over *p*H range 2 to 8)					
Para (γ) substitution						
p-Aminobenzoic acid	0.0365	..	0.051	0.132	(Ineffective above *p*H 6)	
Isonicotinic acid	(Ineffective over *p*H range 2 to 8)					

concentrations at each *p*H value necessary to inhibit all visible mold growth for 48 hrs. at 37.5° C. The spores used for seeding were from a mixed culture of common molds occurring on foodstuffs, and consisted of representatives of the usual types such as *Aspergillus niger, Aspergillus glaucus, Rhizopus nigricans, Penicillium frequentans*, etc. A temperature of 37.5° C. has been found to serve excellently for these tests. On the plates without inhibitor (media plus buffer) there was always a vigorous growth even in 24 hrs., and in 48 hrs. sporulation was heavy. The media consisted of nutrient agar containing 1% sucrose, and the buffers were mixtures of citric acid and disodium phosphate.

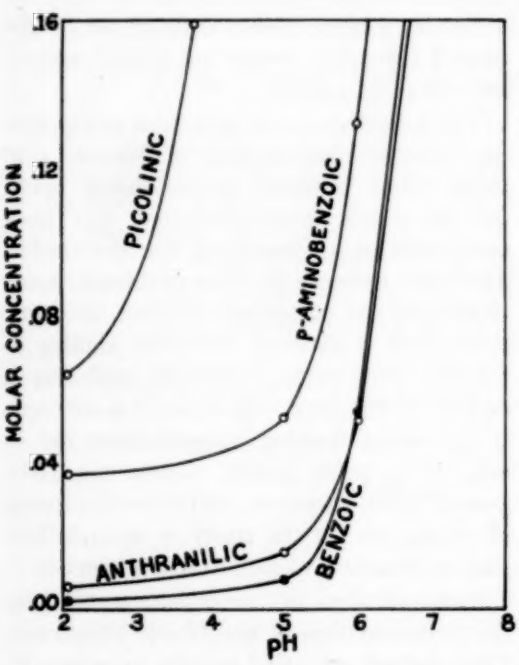

Fig. 1.—Molar Concentrations of the Indicated Acids at Various *p*H Values Necessary to Inhibit Mold for 48 Hours at 37.5° C.

The alpha pyridine carboxylic acid, picolinic, shows fungistatic properties but to a much less degree than benzoic acid. The beta and gamma compounds, nicotinic and isonicotinic,[1] are without mold-inhibiting properties. The action of these compounds is in agreement, therefore, with the previously suggested hypothesis.

The *o*-aminobenzoic acid, anthranilic, is slightly less powerful than benzoic acid, but the decrease in activity is not as much as might be expected from the analogy to salicylic or picolinic acid. *m*-Aminobenzoic acid is a very weak fungistat. At *p*H 2, it inhibits at 0.161 *M*, but even this slight inhibiting power is lost when the *p*H approaches 4. *p*-Aminobenzoic acid, however, presents an interesting contradiction to the expected non-inhibiting nature of such a molecule, since in this compound the two polar groups are present in as widely separated positions as possible.

The fact that *o*-aminobenzoic and *p*-aminobenzoic acids show greater fungistatic properties than would be expected because of the usual effect of the introduction of a polar group into benzoic acid, and because *m*-aminobenzoic acid shows the expected non-inhibiting action, suggests that a condition of resonance masks the polar nature of the amino group in the *ortho* and *para* positions. Buswell, *et al.* (3), state that "one of the important structures in the resonance theory is the quinoid form in which the nitrogen forms a double bond with the carbon of the ring and thereby becomes positive. A nitrogen atom in this condition may readily furnish an acidic hydrogen but

[1] Sample of isonicotinic acid by courtesy of the Upjohn Company.

cannot act as an electron donor since it has no electrons to donate."

The demonstration that the biological activity of a molecule is related to its structural ability to exist as a resonance hybrid is of interest from many points of view. Sulfanilamide, for example, is *p*-aminobenzenesulphonamide. The isomeric *meta* and *ortho* compounds are therapeutically inactive.

REFERENCES

(1) Hoffman, Charles, Schweitzer, T. R., and Dalby, Gaston, *Ind. Eng. Chem.*, 33 (1941), 749–751.

(2) Hoffman, Charles, Schweitzer, T. R., and Dalby, Gaston, *Food Research*, 4 (1939), 539–545.

(3) Buswell, A. M., Downing, J. R., and Rodebush, W. H., *J. Am. Chem. Soc.*, 62 (1940), 2759–2765.

A New Method of Testing Enteric Coatings

By K. Lark-Horovitz and Herta R. Leng†*

It is sometimes necessary to prevent the reaction of medicaments with the gastric juice in the stomach because the substance administered may produce either irritation of the mucous membrane or the preparation may be destroyed by the chemical reaction. To avoid these effects, protective coatings, so-called enteric coatings, are used which are supposed to protect the preparation in the stomach and let it dissolve after a definite length of time in the small intestines. According to Jordan (1) 3.3% of the medicinal preparations on the market are administered enterically, therefore methods to test the efficacy of enteric coatings are of great interest.

To test the coatings in the test tube with solutions artificially reproducing the fluids in the stomach is not very satisfactory or convincing. To investigate the coatings *in vivo*, it is necessary either to make the preparations opaque, so as to produce shadowgraphs with X-rays (2, 3) or to make them self-radiating and to detect their location and behavior by following the radiation. This latter procedure has been proposed by Lark-Horovitz (4). The method is ideally suited for the problem since only a substance which is used in our everyday diet, sodium chloride, is introduced and since the amounts of radiating material are so small as to cause no harm; the lifetime of the radioactive material is so short that the same person can be used again for a test after three or four days.

* Head of the Department of Physics, Purdue University, Lafayette, Ind.

† Amelia Henry Reinhardt International Fellow of the American Association of University Women.

EXPERIMENTAL

Sodium chloride in the form of a crystal is activated by the impact of heavy hydrogen nuclei, deuterons, which are accelerated to several million volts by one of the modern devices available in the nuclear physics laboratories, a cyclotron or high-tension machine. Both the sodium and the chlorine in the salt become radioactive. The lifetime of the chlorine is so short (half-life[1] about one-half hour) that practically one is dealing only with sodium of mass 24, which has a half-life of 14.8 hrs.

The radioactive sodium emits fast electrons and gamma (γ) rays. It is detected mostly by the effect of its γ-rays. For the detection one uses so-called counter tubes which consist of a metallic cylinder and a coaxial wire in a vapor atmosphere, usually argon and a trace of alcohol, at a few centimeters pressure. We have been using Geiger counters consisting of a Dow-metal[2] tube of 11 cm. length, 1.5 cm. width, and a thickness of about 0.01 cm. The counters are filled with an argon-alcohol mixture at about 7 cm. pressure (1 cm. alcohol, 7 cm. argon).

[1] Radioactive substances decay with a certain probability and it is customary to characterize this probability by the time after which half the number of original atoms is still present. Thus radioactive chlorine of mass 38 with a half-life of 37.5 min. has a greater chance to disintegrate than sodium of mass 24 with a half-life of 14.8 hrs.

[2] Since this metal and also aluminum are difficult to obtain at present it is quite feasible to use brass counters with a thin mica window of 0.3 mm. thickness mounted over a grill so that the window will stand the difference between the atmospheric pressure and the vacuum inside.

Usually a NaCl crystal was bombarded with deuterons (of 8 million volts) for 1 microampere hour. This resulted in an initial activity for the sample used of several hundred microcuries (μC).[3] The activated NaCl was scraped from the crystal and 0.3 Gm. was put in a No. 00 gelatin capsule. This was sealed and the enteric coating applied by hand. The enteric coating was prepared in accordance with the formula by Goorley and Lee (2) of the School of Pharmacy of Purdue University and consisted of a mixture of shellac, castor oil and alcohol. To test commercial coatings several capsules of the same type which are to be used for the medicaments are filled with the radioactive material. All of the capsules are run through the coating machine in the usual way and spread out at the end of the process on a tray. Those which are radioactive were picked out with a counter. Since the counting equipment is easily portable it is possible to locate the capsules in the factory without any difficulty.[4]

The actual testing is performed in the following way: The capsule is followed with a counter tube throughout its path in the stomach and intestines. By using a second stationary counter mounted in a lead shield of $4^1/_2$-in. thickness, the distribution in the body is measured by determining the activity of the hand. Any leak of the capsule before the actual process of dissolving of the coating is detected after a few minutes by the activity measured in the hand. As long as the capsule is holding together, it can be followed from point to point with the movable counter. The experiments of Goorley and Lee (2) and Bukey and Klemme (3) have shown that tests are not reliable if the capsule is swallowed during a meal or immediately following. Therefore the experiments were always started after a fasting period of several hours. Some of the capsules remained for hours in the same position in the stomach. In one of our experiments, the capsule was not dislodged for over 10 hrs.; however, the same person tested a few days later showed an extremely short emptying time of $1^1/_2$ hrs. The average emptying time in our experiments was found to be 3 7 hrs. It is interesting to note that the process of solution of enteric coatings in the small intestines in many cases takes place extremely rapidly so that the activity in the intestinal tract suddenly spreads over a wide area and the counts in the hand increase rapidly. In some experiments, the capsule is dissolved slowly and the amount absorbed apparently increases in time.

Ordinary sealed gelatin capsules are dissolved in the stomach after 10 to 20 min. and the absorption curve as measured by the activity of the hand is the same as observed when the salt is administered in aqueous solution.

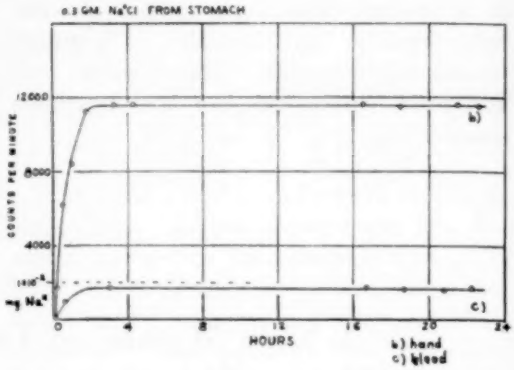

Fig. 1.—Uptake of Radioactive Sodium from the Stomach.

The number of counts per minute is plotted against time in hours indicating the uptake of radioactive sodium from the stomach. A few minutes after intake the sodium can be detected in the hand, reaches equilibrium in a few hours, and remains at this level for 3 to 4 days. Curve *b* represents the activity in the hand; curve *c* represents the activity in the blood. A typical test for enteric coatings is then performed in the following way. The movable counter is used to locate the capsule and the activity in the hand indicates any leak or break in the capsule.

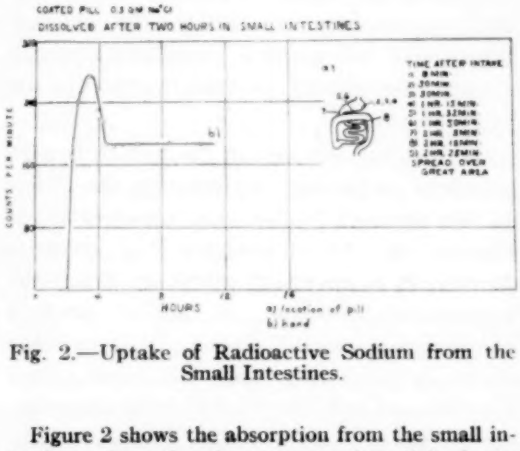

Fig. 2.—Uptake of Radioactive Sodium from the Small Intestines.

Figure 2 shows the absorption from the small intestines. Curve *b* again represents the activity in the hand; Fig. 2 (*a*) shows the location of the capsule at various times. Up to the time the capsule is intact, the activity in the hand is zero. As soon as the capsule dissolves, a rapid increase of the activity in the tissues is observed by measuring the activity in the hand, and sometimes after a period of several hours this high level recedes to the normal level as observed after intake from the stomach.

---

[3] It is customary to give all activities in curies by defining as equivalent to one curie a radioactive sample which emits 2.22×10^{12} counts per minute. A microcurie is then the millionth part or 2.22×10^6 per minute, a quantity which is far too small to cause any biological disturbance, but ample to be detected when distributed throughout the body (6, 7, 8).

[4] Such a portable counting equipment is easily constructed at a cost not exceeding $50 to $100, depending on the equipment used and the precision required.

Table I

	Total No. of Capsules	Dissolved in Intestines	Dissolved in Stomach	Unknown	Not Dissolved
Hand-coated capsules (radioactive method)	23	15 (65.2%)	2 (8.7%)	4 (17.4%)	2 (8.7%)
Commercial coatings (radioactive method)	10	5 (50%)	4 (40%)	1 (10%)	2 (8.7%)
Tests with X-ray method (Goorley and Lee)	137	89 (65%)	4 (3%)	...	44 (32%)

In all the experiments with Na a rapid increase followed by a decrease in activity is observed only if absorption is taking place from the small intestines. It is, therefore, possible to distinguish between the uptake of Na from the stomach and the small intestines by inspecting the activation curve alone. We believe, however, that for diagnostic purposes, such as detection of obstructions of the intestinal tract, the location of the capsule with the movable counter is of interest and importance.

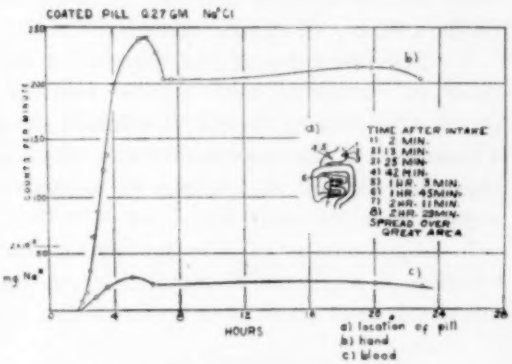

Fig. 3.—Uptake of Radioactive Sodium from the Small Intestines.

Figure 3 shows a curve obtained when a capsule does not break suddenly but dissolves slowly. Also in this case, the maximum in the absorption curve characteristic for the uptake from the small intestines is observed.

As a control of the uptake in the hand, blood activities have also been determined and are shown in figures under *c*. In this way it is possible by comparing the activities to determine whether the uptake from the stomach and from the intestines is of the same order of magnitude. If the active salt is fed from solutions an accurate determination of the concentration can be made by comparing the measured activities with the activity of the original solution. When the salt is taken in capsules, this determination is uncertain as the comparison sample has to be obtained by weighing the dry salt and it is difficult to obtain a uniform mixture of the radioactive and inactive fractions of the salt.[5] Within the limit of error, the final total uptake is the same in both cases, absorption from the stomach or from the small intestines. Corresponding to the maximum uptake in the hand, there is a maximum temporary retention in the blood.[6]

DISCUSSION OF RESULTS

Table I shows the results obtained in the tests made.

The object of these experiments has been to show the effectiveness of the method used and not to accumulate extensive data. For this reason, the number of capsules tested was small as compared with those used in the investigations of Goorley and Lee. The results, however, are in good agreement with their findings. They found that 65% were dissolved in the small intestines as compared to the 65.3% found by us. As stated in their paper, their results of 32% not dissolved represent all incomplete observations, including all cases in which the capsule does not leave the stomach in three hours. Our results give actual observations extended over a two-day period. Whenever a capsule has dissolved, the radioactivity can be observed the following day and, therefore, it is possible to state definitely whether it was dissolved or has passed through the intestinal tract undissolved. Under "unknown," we have listed cases where the capsule dissolved but so long after the intake that it was still in the stomach when the patient left and the activity observed the following day indicated only that disintegration had taken place in the intestines or in the stomach. If we add our "unknown" and "not dissolved" we obtain 26% as compared to the 32% found by Goorley and Lee.

The advantage of this method as compared with the X-ray method is the inexpensive and simple equipment. Besides it also has the advantage of detecting by the activity of the hand any leak or break in the capsule, which is impossible with the X-ray method.

In our note in *Nature* (4), for instance, we have reproduced a curve which shows an activity in the hand from the start, in spite of the fact that the capsule is holding together and can be perfectly well located. This is apparently due to a leak and possibly also to a contamination of the enteric coating during the preparation. The curve shows, first, the slow increase and the level reached due to "absorption from the stomach," and indicates later the sudden break of the capsule in the small intestines

[5] Control measurements of the comparison sample of the powder show that the different mixtures can vary as much as 10% to 15%.

[6] It is important to note that this correspondence between blood and hand values does not exist in the uptake of K. The maximum in the hand is also found, but the blood values remain constant.

and the final level reached after the total salt is absorbed.

The method also allows one, as already mentioned above, to state definitely whether the capsule has dissolved or not, regardless of whether the patient is present at the time the disintegration has taken place. It is not possible to locate the capsule so accurately with a counter as it can be located with the help of X-rays, but that is not necessary for the purpose of testing the efficacy of enteric coatings.

Our experiments show that the formula used by Goorley and Lee is superior to the one particular commercial coating which we have tested. Since we have used hand coating, it is impossible to say whether the commercial method or the composition of the coating is responsible for this difference.

It will be of interest to compare the effect which the different fillings may have on the coatings. This can be done in a simple manner by mixing active NaCl powder with the medicaments to be used. For the pharmacologist and the medical profession, it would of course be of the greatest interest if activated medicaments could be used to study the different rates of absorption from the intestines and from the stomach. This would be possible by synthesizing medicaments containing artificially radioactive atoms. Such an investigation is limited to substances which have a sufficiently long lifetime to permit the complete synthesis to be carried out comfortably. C 14,[7] P 32, S 35, Ca 41, Fe 59, As 74, Sr 89, I 131, are among the elements which could be used. We desire to emphasize, however, that in most of these cases radioactive NaCl would have to be added to make the location of the capsule possible since most of the substances mentioned have radiations too weak to be detected through the tissues.[8] If this addition were made, it would be possible to study systematically the difference in the rates of absorption in the stomach and in the intestines by introducing the medicaments in enteric coatings, locating them by the radiation of NaCl and determining from small samples of ashed tissues and the blood the amount which had been taken up.

[7] The numbers indicate the masses of the respective radioactive elements.

[8] Since the lifetime of all the substances mentioned is far longer than that of Na, these measurements can be comfortably carried out after the Na has decayed. Such experiments shall be carried out in the near future when higher currents in the Purdue cyclotron will be available.

SUMMARY

A new method has been described for testing the efficacy of enteric coatings.

1. Fillings of radioactive NaCl are used in capsules enterically coated and their location is determined by the use of a movable Geiger-Müller counter.

2. This method makes it possible to detect, not only the location of the capsule, but also any possible leak in passing through the intestinal tract by measuring simultaneously the activity in the hand.

3. Comparison of these tests with the results from X-ray investigations substantiates the findings of Goorley and Lee as to the efficacy of enteric coatings.

4. Measurements of the activity of the hand as compared with similar measurements after feeding the salt in solution show a maximum in the absorption curve which is characteristic for the uptake of activated sodium from the small intestines.

5. Applications of this method to the solution of problems of metabolism in the intestinal tract have been pointed out.

REFERENCES

(1) Jordan, C. B., JOUR. A. PH. A., 20 (1931), 930.

(2) Goorley, J. T., and Lee, C. O., *Ibid.*, 27 (1938), 379–384.

(3) Bukey, F. S., and Klemme, C. J., *Ibid.*, 28 (1931), 87–90.

(4) *Nature*, 147 (1941), 580.

(5) Crane, H. R., *Phys. Rev.*, 56 (1931), 1243–1244.

(6) Mullins, Lorin J., *Phys. Rev.*, 56 (1939), 1244.

(7) Hevesy, G., *Phys. Rev.*, 57 (1940), 241.

(8) Goudsmit, *Science*, December, 1939.

Determination of Iodide in the Presence of Interfering Substances*

Kimio F. Shimizu† and Edgar A. Kelly‡

Inorganic iodides in simple solution may be accurately and conveniently determined, but the determination of iodides in heterogeneous mixtures has long been a source of difficulty. Since Pills of Ferrous Iodide, N. F. VI, contain troublesome vegetable material and have no recognized method of assay, this preparation was selected to serve as basis for the development of a procedure which would enable satisfactory determination of iodide in this and other complex mixtures. The work presented here is divided into two parts: first, investigation of efficiency of methods proposed in the literature; and, second, development of a practical and accurate quantitative procedure as applied to ferrous iodide pills.

EXPERIMENTAL

PART I

Standard Reference.—In order to evaluate the efficiency of existing methods of assay, mass of ferrous iodide of known iodide content was used as reference. The mass was freshly prepared before each determination in the following manner:

One-half gram of iodine was accurately weighed in a glass-stoppered weighing bottle, and to it were added 0.4 Gm. of reduced iron and about 1 cc. of distilled water. The weighing bottle was quickly stoppered and rapidly rotated until the pale green solution of ferrous iodide formed. The following previously mixed powders were then added and incorporated: glycyrrhiza, 0.4 Gm.; sugar, 0.4 Gm.; extract of glycyrrhiza, 0.1 Gm.; and acacia, 0.1 Gm.

Standard sodium thiosulfate solution, required in assays to follow, was standardized against iodine taken from the same container as that used in the preparation of the mass. This obviated the necessity of determining the purity of iodine and assured an experimental recovery equal to the theoretical amount.

Two methods, that of François and Lormand (1), and the method of Lansberg (2), were not tested because the former was admittedly lacking in accuracy and the latter, which required extraction in a Soxhlet, filtration, and distillation, appeared to be too laborious and time-consuming to be practical. Following are summaries of those methods which were applied to the analysis of the standard reference.

Method of Fullerton, Watkins and Graham (3).—Weigh enough of the freshly powdered material to represent approximately 5 grains of potassium iodide (or an equivalent amount of iodine), and transfer to a separatory funnel. Add 50 cc. of water and, if alkaline, neutralize with phosphoric acid, finally adding 5 cc. in excess. Add 25 cc. of hydrogen peroxide and agitate thoroughly. Allow to stand a few minutes to be sure the reaction is complete and extract the liberated iodine with several portions of chloroform until the iodine has been removed, as can be told from the color. Collect the chloroform extracts in an iodine flask containing 4 Gm. of potassium iodide in 25 cc. of water. Titrate with 0.1 *N* sodium thiosulfate solution, using starch indicator.

Method of Sheringa (4).—Triturate 10 pills with water and make alkaline with sodium hydroxide solution. Transfer to a 100-cc. volumetric flask with the aid of water and dilute to the mark. Filter and to a 20-cc. aliquot of the filtrate add 50 cc. of water, 10 cc. of dilute sulfuric acid, 7 cc. of 0.1 *N* silver nitrate and 2 cc. of ferric alum indicator. Titrate the excess of silver nitrate with 0.1 *N* potassium thiocyanate.

Method of Kolthoff (5).—Triturate 10 pills with water and rinse into a 100-cc. volumetric flask. Add 2 to 5 cc. of 0.1 *N* sodium thiosulfate followed by 20 cc. of 0.2 *N* barium hydroxide solution; dilute to exactly 100 cc., allow the precipitate to settle, and decant the supernatant liquid through a filter. To a 10-cc. aliquot of the filtrate add 5 to 10 cc. of 4 *N* phosphoric acid and an excess of freshly prepared calcium hypochlorite solution, dilute with about 100 cc. of water and boil off the excess chlorine. Cool, add 5 cc. of 1 *N* potassium iodide solution and titrate the liberated iodine with 0.1 *N* sodium thiosulfate.

Each of the procedures was applied to analysis of standard mass of ferrous iodide. The results of the analyses, recorded in Table I, show that the existing methods gave neither accurate nor consistent recoveries of iodine and they were, therefore, unsatisfactory for the determination of iodide in mixtures.

Modified Kolthoff's Method.—The discordant and low recoveries of iodine with Kolthoff's method were thought to be due to the fact that the precipitate obtained upon the addition of barium hydroxide,

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Table I.—Recovery of Iodine by Various Methods of Analysis

Method	No. of Detns.	Iodine Weighed Mean	Iodine Weighed Range	Iodine Recovered Mean	Iodine Recovered Range	Per Cent Error Mean	Per Cent Error Range
Fullerton	5	0.3005	0.3827–0.2163	0.2922	0.3754–0.2065	3.0	1.5–4.8
Sheringa	6[a]	0.4693	0.5121–0.4051	0.4606	0.5013–0.3934	1.9	1.0–3.1
Kolthoff	7[a]	0.4433	0.5285–0.3435	0.4314	0.4996–0.3278	2.7	1.5–5.5

[a] Each determination checked by duplicate.

Table II

Method	No. of Detns.	Iodine Weighed Mean	Iodine Weighed Range	Iodine Recovered Mean	Iodine Recovered Range	Per Cent Error Mean	Per Cent Error Range
Modified Kolthoff	6[a]	0.4890	0.5375–0.4289	0.4856	0.5326–0.4264	0.6	0.5–0.9

[a] Each determination checked by duplicate.

plus other insoluble material, was not removed before the volume was diluted to 100 cc. Since the volume occupied by the insoluble matter was relatively large and varied in amount with each determination, the aliquot did not represent a proportionate part of the whole. Also, gelatinous precipitates are known to cause serious coprecipitation of ions; it is, therefore, possible that low and inconsistent recoveries were due to adsorption of iodide by the precipitate. With these thoughts in mind, Kolthoff's procedure was modified as follows:

Mass of ferrous iodide (corresponding to the pills) was quantitatively prepared and rinsed into a beaker. After the addition of 2 to 5 cc. of 0.1 *N* sodium thiosulfate and 20 cc. of 0.2 *N* barium hydroxide, the mixture was filtered, receiving the filtrate in a 250-cc. volumetric flask. The beaker and precipitate were washed with successive portions of distilled water until a test portion showed no indication of iodide. The filtrate and washings were then diluted to the mark. A 25-cc. aliquot was taken and treated as described in the original method.

Results of analyses, recorded in Table II, show that the change in procedure produced recoveries which were more uniform and nearer the theoretical amounts. The revised method, however, was time-consuming because the gelatinous character of the precipitate made filtration exceedingly slow and tedious and repeated washings were required to remove the last trace of iodide. Use of suction was not practical because of frothing.

PART II

Results of analyses have shown that earlier methods for the determination of iodide in heterogeneous mixtures are unsatisfactory. We have here attempted to devise a procedure which would permit accurate and concordant recoveries of iodine and at the same time avoid expenditure of excessive time and labor.

Scott (6) gives a method for estimation of iodine by addition of hydrogen peroxide to a solution acidulated with phosphoric acid and subsequent distillation of the iodine into a series of two absorption vessels, each containing a solution of potassium iodide. Serious difficulties were encountered when this method was applied to analysis of mass of ferrous iodide. First, a portion of the iodine condensed in the neck of the distillation flask and could not be recovered; second, all exposed rubber fittings were discolored by iodine; third, steam caused vaporization of iodine from the absorption vessels; and, fourth, excessive frothing of the reaction mixture made quantitative distillation impossible.

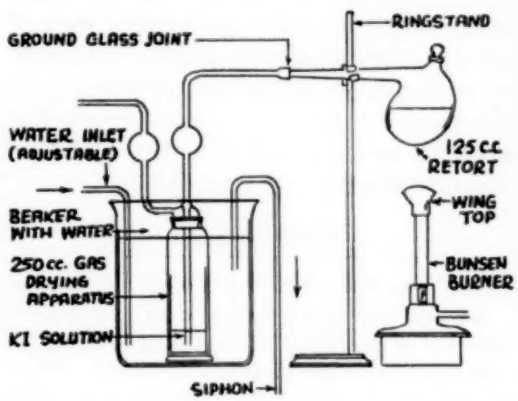

Fig. 1.—Apparatus for Quantitative Distillation of Iodine.

Modifications in the apparatus and procedure overcame the difficulties. A retort having a ground glass opening was substituted for the distillation flash. A Dreschel gas drying apparatus made an excellent receiver and eliminated the necessity of rubber stoppers. A ground glass joint was made to connect the retort to the receiver. Volatilization of iodine from the receiver was prevented by immersing the receiver in a beaker of cold, circulating water. After many futile attempts to overcome or reduce frothing of the boiling mixture, it was found that ferric chloride (or ferric alum) in dilute sulfuric acid was efficient in this respect. The ferric salt, at the same time, served as a mild oxidant for the iodide. Details of the set-up are shown in Fig. 1. The procedure is as follows:

Transfer quantitatively prepared mass of ferrous iodide (or 10 pills of ferrous iodide, if this preparation is to be assayed) to the retort and dilute with water to about 40 cc. Add 10 cc. of 10% sulfuric acid and heat gently to dissolve excess iron. Allow the retort to cool somewhat and connect the retort by means

of the ground glass joint to the Dreschel gas drying apparatus containing 30 cc. of 20% potassium iodide solution. Keep the receiver cold in a beaker of circulating water. Add an excess of ferric chloride (about 5 Gm.) to the retort and gently heat to boiling. Continue the distillation for about 30 min. in such manner as to cause the gases to bubble continuously into the potassium iodide solution. Disconnect the apparatus at the ground glass joint, wash the delivery tube with water and titrate the liberated iodine with 0.1 *N* sodium thiosulfate solution using starch indicator.

The accuracy of the method is evidenced by recoveries of iodine recorded in Table III.

Table III.—Recoveries of Iodine

Detn.	Iodine Weighed	Iodine Recovered	Per Cent Recovery
1	0.3937	0.3914	99.42
2	0.2814	0.2813	99.96
3	0.4483	0.4480	99.93
4	0.3169	0.3162	99.78
5	0.3916	0.3905	99.72
6	0.4396	0.4393	99.93
7	0.3608	0.3589	99.61
8	0.3627	0.3615	99.67
9	0.4408	0.4406	99.95
10	0.3449	0.3448	99.97
11	0.4379	0.4383	100.09
		Average	99.82

Blank determinations were made using the same procedure but with omission of the iodine. Results showed that the vapors from the flask had very little or no reducing action upon the potassium iodide in the receiver. Other experiments were made showing that gelatin (commonly used as pill coating) did not decrease the efficiency of the method.

Table IV.—Analysis of Commercial Brands of Pills of Ferrous Iodide

Company	No. of Detns.	Labeled FeI_2, Gm. per 2 Pills	Determined FeI_2, Gm. per 2 Pills
P. D. & Co.	3	0.122	0.125
Foreign	3	0.09	0.10
Upjohn	3	0.067	0.065

The procedure was applied to analysis of commercial brands of ferrous iodide pills and gave results recorded in Table IV.

SUMMARY

The efficiency of methods proposed in the literature for determining iodide content of mixtures was determined by applying the procedures to analysis of quantitatively prepared mass of ferrous iodide. None of the methods gave accurate or consistent recoveries of iodine.

Kolthoff's method was modified to give satisfactory recoveries of iodine, but the procedure was time-consuming.

A method for the determination of iodide in the presence of interfering substances has been developed by the authors. It is based on the release of iodine with ferric chloride in a specially designed distillation apparatus. The procedure proved to be convenient and it gave accurate recoveries of iodine from samples of quantitatively prepared mass of ferrous iodide.

REFERENCES

(1) François and Lormand, *J. pharm. chim.*, 9 (1914), 332–341; through *Chem. Abst.*, 8 (1914), 2601.

(2) Lansberg, L. M., *Pharm. Weekblad*, 59 (1922), 995–997; through *Chem. Abst.*, 16 (1922), 4006.

(3) Fullerton, J. B., Watkins, W. J., and Graham, C. L., JOUR. A. PH. A., 27 (1938), 417–419.

(4) Sheringa, K., *Pharm. Weekblad*, 61 (1924), 343–345; through *Chem. Abst.*, 18 (1924), 1730.

(5) Kolthoff, J. M., *Pharm. Weekblad*, 59 (1922), 1100–1103; through *Chem. Abst.*, 17 (1923), 324.

(6) Scott, "Standard Methods of Chemical Analysis," Fourth edition, Vol. I.

A Study of Antiseptics in Various Ointment Bases*

By Eileen Foley† and C. O. Lee‡

As early as 1881 Koch observed that phenol, when dissolved in oil or alcohol, showed less bactericidal action than it did in aqueous solution. In 1895 Breslauer (1) reported upon the bactericidal effects of ointments, and concluded that cold cream and lanolin were the best ointment bases for antiseptics. A few years later Eldred (2) showed the presence of bacteria or molds in thirty of the fifty samples of official ointments and bases which he tested.

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In 1929 Reddish and Wales (3) tested 12 ointments of the U. S. Pharmacopœia and 14 of the National Formulary, and found that only five of the former and six of the latter ointments exhibited antiseptic properties. They found that calamine ointment which contained 17% zinc oxide in a base composed chiefly of lard was antiseptic, but that zinc oxide ointment, 20%, in a paraffin and petrolatum base showed no antiseptic action. They concluded that because of the influence exerted by the base it was impossible to predict the antiseptic action of an ointment from the antiseptic value of its constituents.

Rose (4) showed that ointments of ammoniated mercury, iodine, thymol iodide, yellow oxide of mercury, mercury, 30%, and calomel were bacteriostatic, while those of sulfur, zinc oxide, compound resorcinol, ichthyol and tar were not.

Gershenfeld and Miller (5) found that self-emulsifying wax and water-miscible vanishing cream bases were satisfactory vehicles for antiseptics. At about the same time Craw and Lee (6) showed that ointment of phenol, 2%, was not bacteriostatic in a variety of bases, but that the halogen derivatives of *o*-phenylphenol in concentrations as low as 1%, in the same bases, had a marked bacteriostatic action. They concluded that the antiseptic action of ointments is dependent quite as much upon the nature of the chemical as upon the composition of the base.

Prout (7) and his co-workers prepared a series of antiseptic ointments using silica gel as the base. He stated that "there are significant differences, between the individual antiseptic medicaments, which would be expected inasmuch as the germicidal power of the medicaments *per se* varies greatly."

Gershenfeld (8) has proposed the following classification of ointment base groups, according to the size of the zone of inhibition, upon being tested in the usual manner: (*a*) synthetic wax base, (*b*) oxycholesterin base, (*c*) benzoinated lard, (*d*) U. S. P. XI ointment, and (*e*) petrolatum base. Fiero (9) has shown that a partially hydrogenated oil base is superior to the official ointment base for ointment of ammoniated mercury.

EXPERIMENTAL

This investigation has been made for the purpose of studying the antiseptic action of various commonly known chemicals in bases of various types: fatty, non-fatty, water-absorption and gels.

Staphylococcus aureus was the test organism used in this study. The U. S. Department of Agriculture Agar-Plate and Agar-Cup-Plate methods were used, as outlined in Circular No. 198.

Inasmuch as there has been some discussion over the efficacy of wool fat-petrolatum-wax mixtures, in varying proportions, as bases for antispetic ointments a series of such were tested with phenol, 2%, and ammoniated mercury, 10%. Table I shows the ointment formulas and the results of the antiseptic tests. The 2% phenol ointments were consistently not bacteriostatic. The results are shown here merely to contrast them with the positive results of the ammoniated mercury formulas.

Table I

	Base			Antiseptic Action	
No.	Hydrous Wool Fat, Gm.	White Petrolatum, Gm.	White Wax, Gm.	Ammoniated Mercury, 10%	Phenol, 2%
1	10	90	0	6[a]	..
2	20	80	0	6	..
3	30	70	0	6	..
4	40	60	0	6	..
5	50	50	0	6	..
6	60	40	0	6	..
7	70	30	0	6	..
8	80	20	0	6	..
9	90	10	0	6	..
10	10	85	5	6	..
11	20	75	5	6	..
12	30	65	5	6	..
13	40	55	5	5	..
14	50	45	5	6	..
15	60	35	5	5	..
16	70	25	5	5	..
17	80	15	5	5	..
18	90	5	5	5	..

[a] Zones of inhibition in mm. The average of three or more plates.

A second series of 18 formulas was prepared substituting anhydrous wool fat for the hydrous product. Similar antiseptic tests were made with comparable results except that the zones of inhibition averaged about 1 mm. less for the ammoniated mercury ointments. It is possible that this variation may be attributed to the difference in the water content of the two wool fats. The phenol ointments showed no such variation.

WATER HOLDING BASES

A number of workers have proposed several so-called water holding bases as vehicles for antiseptic ointments. It has been stated that bases of this type are more compatible with the skin, especially

Table II

Base or Ointment	Ointments, Official Strength: Boric Acid Mm.[a]	Ammoniated Mercury, Mm.	Yellow Oxide Mercury, Mm.	Calomel, Mm.	Phenol, Mm.	Iodine, Mm.	Whitfield's, Mm.	Ichthammol, Mm.	Calamine, Mm.	Zinc Oxide, Mm.
U. S. P. ointment	..[b]	6	5	0	..	10	0	0	0	..
N. F. ointment	0[c]	0	0	3	0	0	4	3	3	..
Petrolatum	..	5	5	1	..	10	4	3	..	..
Hydrous wool fat	..	7	5	3	..	15	6	3	1	..
Anhydrous wool fat	..	6	4	3	..	14	6	2	..	..
Aquaphor	..	6	5	1	..	20	4	0	..	..
Aquaphor-Water *aa*	4	6	5	3	..	*I*[d]	7*I*	4	..	..
Base 37	..	6	5	1	..	15	4	0	3	..
Base 37-Water *aa*	4	6	5	3	..	*I*	7	4	5	..
Neutramul	0	6	6	0	..	*I*	0	0	0	..
Neutramul-Water *aa*	0	6	6	0	0	*I*	0	0	0	..
Gardinol Base	0	6	0	0	..	0	0	0	0	..
133 T (Goldschmidt Corp.)	0	0	5	0	1	0	0	4	..	..
Seltzer Base 4-Water *aa*	0	0	0	0	4	0	0	0	0	..
Vanishing cream[e]	5	10	8	4	3	*I*	7	4	2	2
Sodium alginate, 5%[e]	9	0	8	4	2	*I*	*I*	*I*	2	2
Methyl cellulose 1500, 5%[e]	9	6	8	4	0	*I*	*I*	*I*	2	2
Methyl cellulose 1500, 15%[e]	0	6	0	3	3	*I*	0	*I*	0	0

[a] Width of zones of inhibition in millimeters.
[b] Negative results.
[c] No test made.
[d] Incompatibility.
[e] Controls showed zones of inhibition of 1 to 2 mm.

so with irritated or exposed areas, and permit more prompt and thorough diffusion of the antiseptic medicament from the base to the affected part.

It is not possible to duplicate conditions *in vitro* as they may be found *in vivo* but certain comparisons can be made. In Table II the zones of inhibition of eight commonly accepted antiseptics in ointment form are shown. Ointments of calamine and of zinc oxide are also shown. The latter gave essentially negative results, but is reported by way of contrast with calamine.

The formulas for several of the proposed new type bases need to be given in order to get a comparison of their value as vehicles for antiseptic ointments. Base 37, proposed by Johnston and Lee (10), is as follows:

Cholesterol	5 Gm.
Anhydrous wool fat	20 Gm.
Liquid petrolatum	45 Gm.
Cetaceum	25 Gm.
White wax	5 Gm.

Seltzer (11) has proposed the following formula for Neutramul:

Sodium lauryl sulfate	2 Gm.
Cetyl alcohol	12 Gm.
Stearyl alcohol	6 Gm.
Ceresin, white	5 Gm.
Liquid petrolatum, heavy	45 Gm.

Burnside and Kuever (12) offer the following formula for Gardinol Ointment Base:

Gardinol	0.25 Gm.
Propylene glycol	6.00 Gm.
Water	1.92 Gm.
White petrolatum	91.83 Gm.

The Goldschmidt Corporation (13) suggests the following formula for an all-purpose cream:

Tego stearate	4.0 Gm.
Beeswax	9.0 Gm.
Water	49.0 Gm.
Borax	1.2 Gm.
Paraffin	12.0 Gm.
Mineral oil	20.0 Gm.
Petrolatum	4.0 Gm.

Another formula in this series is the one which Seltzer designates as Base 4. It is as follows:

Sodium lauryl sulfate	2.0 Gm.
Cetyl alcohol	12.0 Gm.
Stearyl alcohol	3.0 Gm.
Oleyl alcohol	3.0 Gm.
Ceresin, white	5.0 Gm.
Liquid petrolatum, heavy	45.0 Gm.

The vanishing cream formula used is as follows:

Stearic acid	32.00 Gm.
Oil peach kernel	24.00 Gm.
Cetaceum	4.00 Gm.
Glycerin	9.00 Gm.
Ammonia water	4.44 ml.
Hot water	148.00 ml.
Bay rum	25.00 ml.
Oil orange flowers	.50 ml.
Oil lavender	.10 ml.

The methyl cellulose, 5% and 15%, and the sodium alginate, 5%, were gel-like products to which the medicinal agent was added. These were tested because of the interest which has been shown in substances which might serve as non-fatty ointment bases.

SUMMARY AND CONCLUSIONS

Boric acid was negatively bacteriostatic in the fat-like bases but positive in the hydrophilic compounds in which large amounts of water were present.

Ammoniated mercury was consistently active in all of the bases, but most active in vanishing cream, and slightly more active in hydrous wool fat than in the anhydrous fat.

Yellow oxide of mercury gave positive reactions in all the bases used, with the largest zones of inhibition in vanishing cream, sodium alginate and methyl cellulose. Since the controls for these three bases gave zones of inhibition of 1 to 2 mm., the increased zones with them are not particularly significant.

Calomel was, in general, more active in the hydrophilic bases but equally active in anhydrous wool fat.

Phenol was inactive in the usual bases, showing most activity in the Seltzer Base 4 with an equal amount of water, and with vanishing cream, sodium alginate, and methyl cellulose, 15%. Discounting the activity of the controls of the three latter products, the results are not very significant.

Iodine, the most active antiseptically of all the chemicals, behaved very well in all of the fat-like bases. It was incompatible with Neutramul and those vehicles containing large amounts of water.

Whitfield's Ointment was more active in the water-holding bases, but in the most highly aqueous ones developed incompatibilities upon standing. Ichthammol behaved in about the same way, but a little less so.

The inactivity of zinc oxide in all of the ordinary bases was quite in keeping with our past experience. The small zones of inhibition shown with vanishing cream, sodium alginate and methyl cellulose are not significant. The activity of calamine, erratic as it was, is puzzling.

At first glance the results of this study would seem to present a good case in favor of the water-holding bases as vehicles for the antiseptic chemicals. Upon closer examination it will be observed that ammoniated mercury, yellow oxide of mercury, calomel, iodine, benzoic and salicylic acids, ichthammol and even prepared calamine were all bacteriostatic as official ointments. The antiseptic action of each was not greatly accelerated when incorporated in the water-holding bases such as Aquaphor, Base 37, Neutramul, and similar products.

The results with boric acid in the highly hydrated water-holding bases are interesting and perhaps deserving of further study.

It may be said that the presence of water in some of the bases seemed to increase the diffusion of the chemicals into the agar, resulting in larger zones of inhibition. The results were not consistent, however, for all of the chemicals tested.

Therefore, in general, it may be concluded from the results of these experiments that the nature of the chemical or medicament which is incorporated in a base has as much or perhaps more to do with the bacteriostatic action of the product than does the composition of the vehicle in which it has been incorporated.

REFERENCES

(1) Breslauer, E., *Z. Hyg. Infektionskrankh.*, 20 (1895), 165–197.

(2) Eldred, F. R., *Proc. Indiana Pharm. Assoc* (1898), pp. 38–41; PROC. A. PH. A., 47 (1899), 409–410.

(3) Reddish, G. F., and Wales, H. W., JOUR. A. PH. A., 18 (1929), 576–578.

(4) Rose, E. S., *Am. J. Pharm.*, 101 (1929), 52–55.

(5) Gershenfeld, L., and Miller, R. E., *Ibid.*, 105 (1933), 194–198.

(6) Craw, R. O., and Lee, C. O., *Pharm. Arch.*, 9 (1938), 1–16.

(7) Prout, W. A., Eddleman, M. S., and Harris, R. G., JOUR. A. PH. A., 29 (1940), 372–373.

(8) Gershenfeld, L., *Am. J. Pharm.*, 112 (1940), 281–290.

(9) Fiero, G. W., JOUR. A. PH. A., 29 (1940), 458–460.

(10) Johnston, G. W., and Lee, C. O., *Ibid.*, 29 (1940), 236–239.

(11) Seltzer, L. A., *U. S. P. XII Subcom. No. 13*, Bull. 11 (1941), p. 29.

(12) Burnside, C. B., and Kuever, R. A., JOUR. A. PH. A., 29 (1940), 373–379.

(13) "The Goldschmidt Corporation Leaflet," January, 1940.

The Elimination of Ergotoxine, Ergotamine and Ergonovine*

By Jerome C. Kopel and James M. Dille†

Goodman and Gilman (1) state that "Little is known of the fate and excretion of the ergot alkaloids." This summarizes the general impression which is found from a survey of the literature on ergot. Ordinarily a clue to the fate of the drug can be had by following the rate of elimination of the drug by chemical methods, but the difficulty in this case lies in the fact that such a small quantity of drug is administered as the original dose, that only extremely small quantities of the substance are present in the blood and tissues. In such cases the pharmacological procedure of essential elimination can often be carried out, but in preliminary experiments on frogs, rats, rabbits and white leghorn cocks this was found to be unreliable because of the wide variations in the reactions of the animals to a given dose and because of the occurrence of delayed gangrene often appearing a week after the administration of a single dose. These obstacles caused us to reëxamine the possibility of using chemical methods and it was found that by the adoption of special procedures and the use of guinea pigs which have a relatively high fatal dose it was possible to secure data on the rate of disappearance of the three active alkaloids of ergot from the blood and muscle after the administration of sublethal doses.

EXPERIMENTAL

THE ANALYSIS OF ERGOTOXINE, ERGOTAMINE AND ERGONOVINE IN BLOOD AND TISSUES

Principle of the Method.—This is a colorimetric method based on the production of a blue color with *p*-dimethylaminobenzaldehyde as described by Van Urk (2). This reaction was used for the assay of ergot by Smith (3) and adopted as an official method for this purpose in the 1934 British Pharmacopœia.

This procedure as modified for the purposes of tissue analysis involves the extraction of the alkaloid from blood or tissues with ether, separation of the alkaloid from the ether by shaking out with tartaric acid solution, treatment of this solution with the *p*-dimethylaminobenzaldehyde reagent, and the estimation of the intensity of the blue color in a photoelectric colorimeter. The concentration of the alkaloid is then determined from a previously prepared standard-concentration curve.

Procedure for the Treatment and Extraction of Tissue.—Blood: The blood, citrated or coagulated, is carefully measured and placed in a large porcelain evaporating dish. Two cubic centimeters of concentrated ammonia solution (28%) is added for each 10 cc. of blood. After the mixture has been allowed to stand for five minutes, anhydrous sodium sulfate is added in small portions with constant stirring in the proportion of 20 Gm. of the salt to each 10 cc. of the blood. Fifteen to twenty minutes are allowed for the mixture to dry completely. The dry cake is then broken up by means of a glass pestle and mixed with about 2 Gm. of shredded asbestos for each 30 Gm. of the dry powder. The material is then carefully removed from the dish and transferred to a Soxhlet thimble of the proper size. Extraction is then carried out with ether for five hours. At the end of this time, the ether is removed and saved. The marc is removed from the thimble, again broken up, realkalinized with the original quantity of ammonia, returned to the Soxhlet apparatus, and extracted for five hours with a new portion of ether. The contents of the flask are removed and saved. The procedure is repeated for the third time. The three ether extracts are combined, concentrated on a water bath to about 35 cc., transferred to a separatory funnel and shaken out with a 1% aqueous tartaric acid solution, 2 cc. being added each time for five washings. The combined tartaric acid portions are heated at 40° C. to remove the dissolved ether and made up to a volume of 10 cc. This solution is then ready to be treated as described for the colorimetric determinations.

Other Tissues: The procedure differs from that for blood in that no sodium sulfate is necessary. The tissue is completely ground in a meat grinder, weighed, alkalinized with ammonia and mixed with asbestos. The same proportions are used as in the case of the dried blood and the material is transferred directly to the Soxhlet thimble. Three five-hour extractions are then made with ether as described above. The combined ether extracts are concentrated as described for blood and shaken with several portions of a very dilute ammonia solution which are withdrawn and discarded. This removes extracted fats. The ether is then shaken out five times with 2-cc. portions of a 1% aqueous solution of tartaric acid. The combined tartaric acid portions are heated at 40° C. to remove the dissolved ether and made up to a volume of 10 cc. This solution is then ready to be tested colorimetrically by the following method.

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Colorimetric Determination of Ergot Alkaloids in Extracts of Blood and Tissues.—The 10-cc. volume of tartaric acid extract obtained above is treated by carefully adding 20 cc. of *p*-dimethylaminobenzaldehyde reagent solution to the tartaric acid extract while the temperature of the mixture is kept at 10° C. The reagent is a 0.125% solution of *p*-dimethylaminobenzaldehyde in a 65% sulfuric acid solution containing, in addition, 0.1 cc. of a 5% solution of ferric chloride (British Pharmacopœia, 1934) for each 100 cc. of the reagent. The tartaric acid extract and reagent are thoroughly mixed and allowed to stand in the light for one hour to insure a maximum color and to permit the solution to come to room temperature. At the end of this time, 25 cc. of the blue-colored solution is transferred to the cell of the colorimeter and a reading of the photoelectric current reduction is made. This reading is then referred to the previous prepared graph to give the amount of alkaloid present. From this value the amount of alkaloid recovered is calculated in terms of the alkaloidal salt and can be most conveniently expressed in terms of milligrams per cent.

Evaluation of the Colorimetric Assay.—Specificity: The blue color is specific for the indol group with the 2-position unsubstituted. In lysergic acid this position is free and this gives the characteristic color reaction of the ergot alkaloids (4). It does not distinguish between the individual alkaloids of ergot but this is unimportant for the purposes of this work since only one known alka'oid is administered, but if this colorimetric method is used for toxicological purposes it would not indicate specific ergot alkaloids.

Sensitivity: The sensitivity of the color reaction was fixed by Van Urk at $^1/_{400}$ mg. of ergotamine or ergotoxine. In the preparation of the colorimeter reading-concentration curve the lowest detectable color was found with a concentration of 0.0005 mg./cc. of standard solution of each of the salts of the three alkaloids. This reading was made on a total volume of 25 cc. of standard solution and reagent. Therefore, since 10 cc. of solution is required, the smallest quantity which can be detected by this test is 0.005 mg.

Accuracy: Small quantities of each of the three ergot alkaloids were added to freshly secured blood, muscle, liver and kidney. The tissue was then analyzed by the above procedure. Results are shown in Table I. It will be seen that there is considerable loss in certain cases. About 50% of the alkaloid was recovered from muscle tissue and about 83% from blood. Liver and kidney tissue gave very poor recoveries and were therefore not used in the experiments. While these recoveries are not perfect they can be regarded as satisfactory in view of the small original doses and in view of the checks between recoveries from each kind of tissue. However, in view of these recoveries in control experiments, care was taken to make any interpretations of results on a relative rather than an absolute basis.

Table I.—The Recovery of Ergotoxine Ethanesulfonate, Ergotamine Tartrate, Ergonovine Tartrate, Added to 10 Cc. of Blood or 50 Gm. of Tissue

Alkaloid	Tissue	Amount Added, Mg.	Amount Recovered, Mg.	Per Cent Recovery
Ergotoxin ethanesulfonate	Muscle	0.300	0.157	53
	Muscle	0.300	0.140	49
	Muscle	0.150	0.078	52
	Muscle	0.100	0.055	55
	Muscle	0.100	0.050	50
	Blood	0.100	0.080	80
	Blood	0.085	0.070	82
	Blood	0.070	0.057	82
	Liver	0.165	0.055	33
	Liver	0.165	0.048	29
	Liver	0.165	0.062	37
	Liver	0.165	0.050	30
	Kidney	0.165	0.040	24
	Kidney	0.165	0.040	24
Ergotamine tartrate	Muscle	0.700	0.330	47
	Muscle	0.700	0.355	51
	Muscle	0.350	0.160	46
	Blood	0.070	0.060	86
Ergonovine tartrate	Muscle	0.700	0.370	53
	Muscle	0.700	0.325	46
	Muscle	0.350	0.175	50
	Blood	0.113	0.097	86
	Blood	0.070	0.057	82
	Blood	0.070	0.060	86
	Blood	0.056	0.450	82

THE RATE OF DISAPPEARANCE OF ERGOTOXINE, ERGOTAMINE AND ERGONOVINE FROM BLOOD AND MUSCLE

Adult male guinea pigs were used in all these experiments. The solutions of the drug were administered intracardially in all cases. No serious difficulty was encountered but if there was any question of faulty technique the animal was not used. The solutions were prepared for injection by patiently triturating the carefully weighed alkaloidal salt in an agate mortar with a small amount of 1% aqueous tartaric acid solution until solution was effected. This was then transferred to a volumetric flask and made up to volume. Solutions of each alkaloid were approximately 0.3% of the alkaloidal salt. The following doses were used: 16 mg. of ergotoxine ethanesulfonate per Kg., 25 mg. of ergotamine tartrate per Kg., and 25 mg. of ergonovine tartrate per Kg.

At the end of the proper time interval after the injection, the animal was rendered unconscious by a blow on the head and killed by exsanguination. Ten to fifteen cubic centimeters of blood was collected in a citrated vessel and used for the anlysis. About 50 Gm. of muscle tissue was obtained from the thigh and saddle. The liver was excised and analyzed for some experiments but the results were not sufficiently reliable to include. The results are summarized in the tables and figures. Each point represents one animal and there is consequent variation due to individual differences, but the curves indicate the general concentration of these three alkaloids in the blood and muscle at various times after administration.

Table II.—Concentration of Ergotoxine, Calculated as Ergotoxine Ethanesulfonate, in Blood and Muscle of Guinea Pigs after the Intracardiac Administration of 16 Mg./Kg. of Ergotoxine Ethanesulfonate

Animal Number	Weight, Kg.	Total Dose, Mg.	Elapsed Time between Administration and Sacrifice of Animal, Min.	Recovery Muscle Mg./100 Gm.	Recovery Blood Mg./100 Cc.
48	0.43	6.88	15	0.14	5.40
53	0.49	7.84	30	0.18	3.80
62	0.64	10.24	60	0.37	0.90
55	0.50	8.0	120	0.24	1.0
65	0.53	8.48	180	0.12	0.23
87	0.46	7.36	240	0.10	0.18
66	0.49	7.84	300	0.09	0.25
70	0.57	9.12	420	0.07	None

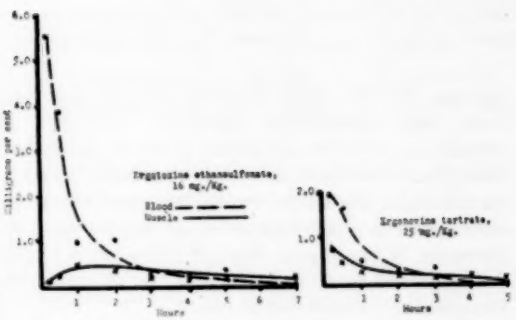

Fig. 1.—The Concentration of Ergotoxine (as Ethanesulfonate) or Ergonovine (as Tartrate) in the Blood and Muscle of Guinea Pigs after the Intracardiac Administration of 16 Mg. of Ergotoxine Ethanesulfonate per Kg. and 25 Mg. of Ergonovine Tartrate per Kg.

Table III.—Concentration of Ergotamine, Calculated as Ergotamine Tartrate, in Blood and Muscle of Guinea Pigs after the Intracardiac Administration of 25 Mg./Kg. of Ergotamine Tartrate

Animal Number	Weight, Kg.	Total Dose, Mg.	Elapsed Time between Administration and Sacrifice of Animal, Min.	Recovery Muscle Mg./100 Gm.	Recovery Blood Mg./100 Cc.
28	0.56	14.00	15	0.16	5.0
22	0.52	13.00	30	0.18	4.0
23	0.62	15.63	60	0.20	3.30
25	0.66	16.50	90	0.21	1.09
44	0.49	12.25	120	0.35	0.63
27	0.59	14.70	180	0.15	0.19
61	0.46	11.50	240	0.285	0.44
56	0.44	11.00	300	0.25	0.38
71	0.46	11.50	420	0.17	None
72	0.53	13.25	720	0.05	None

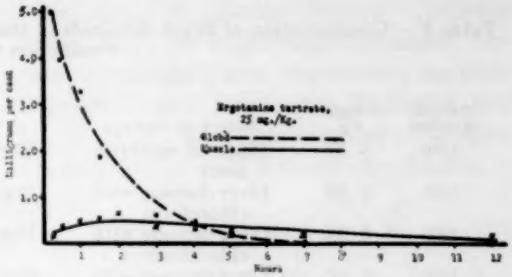

Fig. 2.—The Concentration of Ergotamine (as Tartrate) in the Blood and Muscle of Guinea Pigs after the Intracardiac Administration of 25 Mg. of Ergotamine Tartrate per Kg.

Table IV.—Concentration of Ergonovine, Calculated as Ergonovine Tartrate, in Blood and Muscle of Guinea Pigs after the Intracardiac Administration of 25 Mg./Kg. of Ergonovine Tartrate

Animal Number	Weight, Kg.	Total Dose, Mg.	Elapsed Time between Administration and Sacrifice of Animal, Min.	Recovery Muscle Mg./100 Gm.	Recovery Blood Mg/100 Cc.
59	0.58	14.50	15	0.74	1.91
60	0.53	13.25	30	0.40	1.75
57	0.46	11.50	60	0.12	0.27
36	0.51	13.55	60	....	0.27
38	0.45	11.25	90	0.10	....
88	0.54	13.50	120	0.08	0.17
49	0.48	11.90	180	0.07	0.25
40	0.47	11.75	240	0.06	None
58	0.44	11.00	300	None	None

ROLE OF THE LIVER AND KIDNEYS IN THE ELIMINATION OF ERGOT ALKALOIDS

The animal organism may eliminate a drug by either detoxication or excretion. In the former case the liver is presumed to play a leading role, while the kidneys are the principal organs of excretion. In an attempt to indicate the part these organs play in the elimination of the ergot alkaloids two kinds of experiments were carried out.

Bilateral nephrectomy was performed on three guinea pigs under light ether anesthesia. Twenty-four hours later 16 mg. of ergotoxine ethanesulfonate per Kg. was administered intracardially to one animal and 25 mg. of ergotamine tartrate per Kg. to the other two. After a suitable interval, indicated from the previous experiments, the animals were killed and the blood and muscle analyzed for the alkaloid. Table V shows the results. Comparison of these results to those in Tables II and III shows no significant difference in the blood and muscle concentrations of the alkaloid. Hence it is apparent that the diminution of the alkaloids in the blood and

Table V.—Concentration of Ergot Alkaloids in the Blood and Muscle after Bilateral Nephrectomy or the Production of Liver Damage

Animal Number	Weight, Kg.	Nature of Damage	Drug	Dose, Mg./Kg.	Minutes after Administration	Concentration	
						Muscle Mg./100 Gm.	Blood Mg./100 Cc.
164	0.57	Bilateral nephrectomy	Ergotoxine	16	180	0.10	0.26
182	0.52	Liver damage with chloroform	Ergotoxine	16	180	0.20	0.14
183	0.38	Liver damage with chloroform	Ergotoxine	16	180	0.22	0.25
184	0.42	Liver damage with phosphorus	Ergotoxine	16	180	0.15	0.39
191	0.47	Liver damage with phosphorus	Ergotoxine	16	180	0.49	0.35
193	0.43	Liver damage with phosphorus	Ergotoxine	16	180	0.25	0.46
167	0.59	Bilateral nephrectomy	Ergonovine	25	120	0.075	0.20
168	0.45	Bilateral nephrectomy	Ergonovine	25	120	0.065	0.23
181	0.72	Liver damage with chloroform	Ergonovine	25	120	0.174	0.15
189	0.37	Liver damage with phosphorus	Ergonovine	25	120	1.23	0.38

muscle is not dependent upon the intactness of the kidneys.

Hepatectomy is difficult to perform and is accompanied by undesirable effects from the surgery but fairly complete damage to the liver may be produced by prolonged administration of chloroform or by the administration of phosphorus. Three animals were treated by two hours of chloroform administration and four were administered two doses of 7 mg. of phosphorus in 0.35% solution in olive oil per Kg. subcutaneously twenty-four hours apart. Twenty-four hours after the administration of the hepatatoxic agent, 16 mg. of ergotoxine ethanesulfonate per Kg. was administered to five animals and 25 mg. of ergonovine tartrate per Kg. was administered to two animals. The animals were killed at the end of a definite time and the blood and muscle analyzed for the alkaloid. Results in Table IV indicate that, while there is considerable variation in these values which can be attributed to the difference in the extent of damage produced, in animals with damaged livers the rate of diminution of the ergot alkaloids is slowed and that, therefore, the liver plays a part in the elimination of these alkaloids.

DISCUSSION

The concentration of each of the three active alkaloids in the blood decreases rapidly after administration. This is probably due to the passage of the alkaloid into the tissues because there is a rise of concentration in the muscle during this period. This decrease in blood concentration becomes much less rapid by the end of two hours, and the last period is characterized by a slower rate of decrease. The concentration of the alkaloid in muscle is also decreasing until after seven hours for ergotoxine, twelve hours for ergotamine and five hours for ergonovine the alkaloid has practically disappeared. These times cannot be accepted as absolute, but it is obvious that the alkaloids must disappear from the body with considerable rapidity. This rate of elimination of these alkaloids is not in agreement with Burn (5) who found the blood pressure response to adrenalin the same twenty-four and forty-two hours after oral administration of powdered ergot. However, it agrees with Ifuku (6) who found by the same method that the action of 1 mg. of ergotoxine intravenously lasted eleven hours.

In view of this relatively rapid elimination of these alkaloids it becomes difficult to accept the belief that they are the direct cause of the gangrene which may occur after a single dose but which does not appear for several days or a week. The means by which the ergot alkaloids produce gangrene is not clear. McGrath (7) in verifying the effect of ergotamine in rats, described by Rothlin (8), states that there are symptoms of acute intoxication for two hours, after which the animals appear normal. At the end of the third to the fifth day the tail becomes pale and by the sixth to ninth day is cyanotic with sloughing of the distal portion and healing of the stump by the sixteenth to the twentieth day. Pathological changes are most marked in the small arteries and arterioles. There is cellular proliferation and swelling of the intima with final occlusion of the lumen. The important point, however, is that these extensive pathological changes do not occur until, as we have shown, long after the ergot alkaloid has been eliminated. This leads us to postulate that these alkaloids initiate the changes which result in gangrene and that these changes once started run their course without the necessity of the presence of the alkaloids.

The ergot alkaloids appear to be detoxicated in the body rather than excreted since bilateral nephrectomy results in no significant difference in the concentration at the end of equivalent periods. The liver seems to play a part in the detoxication

since the production of liver damage results in a higher concentration of ergotoxine and ergonovine at the end of selected intervals.

The method described for the determination of ergotoxine, ergotamine and ergonovine in blood and other tissues should be useful for toxicological purposes. The ordinary toxicological texts such as McNalley (9), Peterson, Haines and Webster (10), and Autenrieth and Warren (11) give no method for this purpose if the amount of alkaloid is small. Rosenbloom and Schildecker (12) report that they were able to isolate a crystalline material which gave color reactions and melting point of ergotinine from stomach, intestine, kidney and liver analyzed together. The method described here is sufficiently sensitive but is not specific for a particular ergot alkaloid. The method of drying the blood proved to be so effective that we suggest it for other alkaloids.

SUMMARY

The color reaction of ergot alkaloids with *p*-dimethylaminobenzaldehyde can be used to make quantitative estimation of ergotoxine, ergotamine and ergonovine in blood and muscle.

The three pharmacologically active ergot alkaloids, ergotoxine, ergotamine and ergonovine, disappear quite rapidly from the blood and muscle of guinea pigs after the administration of these drugs. Thus the late appearance of gangrene cannot be explained by the long-continued presence of these drugs in the body.

The alkaloids appear to be detoxicated rather than excreted and the liver appears to take part in this process.

We wish to thank Sandoz Chemical Works, Inc., for supplying ergotamine tartrate (Gynergen) and ergonovine tartrate (Basergin); and Burroughs Wellcome and Co., U. S. A., for supplying the ergotoxine ethanesulfonate used in these experiments.

REFERENCES

(1) Goodman, L., and Gilman, A., "The Pharmacological Basis of Therapeutics," The Macmillan Company, New York, N. Y., 1941.

(2) Van Urk, H. W., *Pharm. Weekblad*, 66 (1929), 473.

(3) Smith, M. I., *Pub. Health Rep.* (1930), p. 1466.

(4) Barger, G., *Hand. der Exper. Pharmakol.*, 6 (1938), 90.

(5) Burn, J. H., *Pharm. J.*, 118 (1927), 565.

(6) Ifuku, D., *Japan J. Med. Sci.*, 8 (1935), 148.

(7) McGrath, E. J., *Arch. Intern. Med.*, 55 (1935), 492.

(8) Rothlin, E., *Arch. intern. de pharmacodynamie*, 27 (1923), 459.

(9) McNalley, W. D., "Toxicology," Industrial Medicine, Chicago, Ill., 1937.

(10) Peterson, F., Haines, W. S., and Webster, R. W., "Legal Medicine and Toxicology," W. B. Saunders Company, Philadelphia, Pa., 1923.

(11) Autenrieth, W., and Warren, W. H., "Laboratory Manual for the Detection of Poisons and Powerful Drugs," The Blakiston Company, Philadelphia, Pa., 1928.

(12) Rosenbloom, J., and Schildecker, C. B., *J. Am. Med. Assoc.*, 63 (1914), 1203.

Strychnine X. Comparative Accuracies of Stomach Tube and Intraperitoneal Injection Methods of Bioassay*

By Justus C. Ward and D. Glen Crabtree

While carrying out the experiments with strychnine, upon which the earlier papers in this series[1] were based, we found it would be desirable to conduct adequate studies to compare our system of stomach tube bioassay with the better recognized intraperitoneal injection method. In using strychnine as an economic poison, it must be mixed with baits to be taken by mouth by predatory animals and rodents. Consequently, we have adopted the stomach tube bioassay procedure to approximate most closely the conditions under which the poison must act under field conditions.

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[1] "Strychnine I," JOUR. A. PH. A., 19 (1930), 954–957; "II," *Ibid.*, 19 (1930), 1057–1060; "III," *Ibid.*, 23 (1934), 984–988; "IV," *Ibid.*, 25 (1936), 422–426; "V," Thesis, University of Colorado; "VI," JOUR. A. PH. A., 25 (1936), 590–593; "VII," *Ibid.*, 26 (1937), 29–31; "VIII," *Ibid.*, 26 (1937), 129–134; "IX," Unpublished manuscript.

We feel that this is a most logical system for evaluating potent drugs and poisons which must be taken by mouth, since a bioassay should indicate definitely the results to be expected from the general use of the material assayed.

To investigate several aspects of strychnine bioassays, we have tested this poison in the form of the alkaloid and as the sulfate. We have administered it by stomach tube and by intraperitoneal injection to both male and female rats. In the present work from 18 to 36 rats have been used per dose. These experiments were completed between September, 1940, and early June, 1941, and a total of 918 mature adult white rats of one uniform strain were used. The technique of stomach tube bioassay employed varied only slightly from that previously reported (1). In the present experiments, the authors worked always together, one of us holding the rats without arousing in them undue nervous excitation, while the other administered the previously measured dose through a No. 8 soft rubber catheter which had been passed into the stomach through a perforated, wooden gag. The same holding technique was used when the intraperitoneal injections were given. In all strychnine alkaloid bioassays, the poison was suspended by first mixing the strychnine with a double quantity of U. S. P. powdered Gum Acacia in the dry condition; a few drops of water were added to this mixture and the mass stirred until all the strychnine particles were completely wetted. The resulting paste was then diluted to such a volume that 1 cc. of suspension contained exactly 1 mg. of strychnine alkaloid. This suspension was always stirred rapidly for a few seconds before each dose was removed. In the strychnine sulfate bioassays a true solution was prepared which carried the equivalent of 1 mg. of strychnine alkaloid per cubic centimeter of solution.

The poison samples used in these experiments were taken from laboratory reference lots which had been tested previously and all found to be equally toxic, and consisted of U. S. P. IX Strychnine Alkaloid and U. S. P. Strychnine Sulfate Crystals manufactured by the same firm.

Our experimental results are shown in Tables I, II, III, IV and V.

Table I.—Toxicity of Strychnine Alkaloid by Stomach Tube

Dose, Mg./Kg.[a]	Male Rats No. Used	Male Rats % Mortality	Female Rats No. Used	Female Rats % Mortality
15.00	18	89	18	100
10.00	18	83	18	100
7.50	18	89	18	100
6.00	18	33	18	100
5.00	18	22	18	100
4.00	..	..	18	95
3.00	..	..	27	74
2.50	..	..	27	70
2.25	..	..	18	33

[a] Milligrams of strychnine per kilogram of animal body weight.

Table II.—Toxicity of Strychnine as the Sulfate by Stomach Tube

Dose, Mg./Kg.[a]	Male Rats No. Used	Male Rats % Mortality	Female Rats No. Used	Female Rats % Mortality
15.00	18	78	18	100
10.00	18	94	18	100
7.50	18	72	18	100
6.00	36	31	18	100
5.00	18	17	18	100
4.00	..	..	18	94
3.00	..	..	18	94
2.50	..	..	18	39

[a] Milligrams of strychnine per kilogram of animal body weight.

Table III.—Toxicity of Strychnine as the Sulfate by Intraperitoneal Injection

Dose, Mg./Kg.[a]	Male Rats No. Used	Male Rats % Mortality	Female Rats No. Used	Female Rats % Mortality
15.00	18	100	18	100
10.00	18	100	18	100
7.50	18	100	18	100
6.00	18	100	18	100
5.00	18	100	18	100
4.00	18	95	18	100
3.00	18	67	18	95
2.50	18	22	18	89
2.25	..	..	18	83
2.00	..	..	18	89
1.75	..	..	18	83
1.60	..	..	18	56
1.50	..	..	18	61
1.40	..	..	18	39

[a] Milligrams of strychnine per kilogram of animal body weight.

Table IV.—Summary of Toxicity of Strychnine to Mature White Rats

	L.D.50% Computed from Mortality Curves	
	Male Rats	Female Rats
Strychnine alkaloid by stomach tube	6.50 mg./Kg.	2.35 mg./Kg.
Strychnine as the sulfate by stomach tube	6.50 mg./Kg.	2.60 mg./Kg.
Strychnine as the sulfate by intraperitoneal injection	2.80 mg./Kg.	1.45 mg./Kg.

In Table V are shown the comparative speeds of kill with strychnine at the different

dosage levels used. Obviously only those animals dying in each series can be listed in such a tabulation, so the per cent mortality at each dose is included to aid in evaluating the data.

Table V.—Speed of Strychnine Action

Dose, Mg./Kg.[a]	Average Time to Death in Minutes: Male Rats % Mortality	Male Rats Time	Female Rats % Mortality	Female Rats Time
A. As the alkaloid by stomach tube				
15.00	89	10.8	100	7.4
10.00	83	12.5	100	7.3
7.50	89	11.9	100	8.5
6.00	33	19.5	100	8.5
5.00	22	13.3	100	8.3
4.00	..	..	95	11.2
3.00	..	..	74	12.0
2.50	..	..	70	14.1
2.25	..	..	33	13.3
B. As the sulfate by stomach tube				
15.00	78	11.0	100	7.2
10.00	94	13.1	100	7.3
7.50	72	14.0	100	7.5
6.00	31	14.8	100	7.5
5.00	17	11.0	100	8.1
4.00	..	..	94	11.1
3.00	..	..	94	12.6
2.50	..	..	39	15.8
C. As the sulfate by intraperitoneal injection				
15.00	100	4.1	100	3.6
10.00	100	5.7	100	3.6
7.50	100	6.1	100	7.3
6.00	100	8.9	100	8.3
5.00	100	8.8	100	6.8
4.00	95	9.5	100	9.4
3.00	67	13.3	95	9.7
2.50	22	19.3	89	11.6
2.25	..	..	83	9.9
2.00	..	..	89	18.5
1.75	..	..	83	18.5
1.60	..	..	56	15.6
1.50	..	..	61	12.2
1.40	..	..	39	12.2

[a] Milligrams of strychnine per kilogram of animal body weight.

DISCUSSION

Tables I, II and III bring out quite forcibly the comparison between stomach tube and intraperitoneal injection methods of bioassay for strychnine. They show how difficult it is to obtain a 100% mortality of male rats with strychnine either as the alkaloid or as the sulfate when it is given by stomach tube at moderate doses. This observation is correlated with field reports of variable results found in using strychnine as an economic poison. They also show that strychnine toxicity follows the general pattern of the standard curve, with the susceptibility of the female rat approaching the ideal in most cases.

Table IV illustrates the differences in toxicity of strychnine to male and female rats. Although this characteristic is more marked for stomach tube administration, it is shown quite plainly on intraperitoneal injection also. Other workers have made similar observations on this sex variation (2).

Table V shows that there are smaller differences in the times required to kill susceptible individuals than the large variations in doses administered would indicate should be expected.

The results of these tests justify our selection of stomach tube bioassay on mature male rats for properly evaluating the efficiency to be expected from the use of strychnine under field conditions (1). They furthermore prove that reliable bioassays can be made using either suspensions of strychnine alkaloid or solutions of strychnine as the sulfate administered by stomach tube.

CONCLUSIONS

1. Strychnine given by stomach tube is uniformly less toxic than it is when given by intraperitoneal injection.

2. Strychnine as the alkaloid and as the sulfate is equally toxic when given by stomach tube to male rats, and it varies only slightly when tested on female animals.

3. Female rats are more than twice as susceptible to strychnine at the $L.D._{50\%}$ dose by stomach tube than are male rats of the same strain.

4. Female rats are slightly less than twice as susceptible as are males to strychnine by intraperitoneal injection.

5. Bioassay of strychnine alkaloid held in suspension by means of 0.2% gum acacia and administered to standardized male rats by stomach tube gives the most accurate indication of the field efficiency to be expected of the sample tested.

BIBLIOGRAPHY

(1) Ward, Munch and Garlough, JOUR. A. PH. A., 25 (1936), 590.

(2) Poe, Suchy and Witt, *J. Pharm. Exptl. Ther.*, 58 (1936), 239.

Effects of Testosterone Acetate and Propionate and of Estradiol Dipropionate upon the Resistance of the Rat to Evipal Sodium, Nostal, Pernoston and Pentobarbital Sodium*†

By Harald G. O. Holck, Donald R. Mathieson, Edwin L. Smith and Lewis D. Fink‡

That prolonged administration of male sex hormone from human urine raises the resistance of female and castrated male rats to the barbiturate, evipal, was first shown by Holck, *et al.* (1). Preliminary reports of similar effects by testosterone esters against several barbiturates were subsequently published (2, 3). Antinarcotic effect of testosterone against pentobarbital in the rat has recently been reported by Kinsey (4). Crabtree, Ward and Welch (5), by administering this hormone, raised the resistance of castrated male rats to red squill to the normal level. Störtebecker (6) concluded that estrogenic hormones increased the resistance of rabbits, guinea pigs and mice to depression by ether, magnesium, alcohol, curare and the barbiturate, pernoston. Also, androsterone elevated the rabbit's resistance to ether anesthesia.

Our present studies are intended to establish the necessary doses and durations of administration of various synthetic sex hormones and the limitations of their action, depending on the barbiturate and species of animals.

EXPERIMENTAL

Our albino rats were originally bred from Wistar stock; the hybrids were from our College of Agriculture (Table IV) or bred from rats presented by Professor Carmichael of the University of Alabama (Table V). The diet consisted of Purina Dog Chow. The rat quarters were kept at approximately 25° C. During the barbiturate tests, each rat was kept alone at 27–28° C. and considered recovered when it retracted either hind foot promptly from an extended position.

In the pentobarbital (8097 x 974417, Lilly) experiments, ten sites of injection were used in rotation and the 1% solution was prepared fresh several times daily and kept well protected during the interim between each series of injections.

PART I. INFLUENCE OF VARYING DOSES AND DURATIONS OF ADMINISTRATION OF TESTOSTERONE ACETATE (PERANDREN) UPON THE RESPONSE OF ALBINO RATS TO SINGLE DOSES OF PERNOSTON (SODIUM 2-BUTYLBROMALLYL BARBITURATE) AND TO SODIUM EVIPAL (SODIUM METHYL-CYCLOHEXENYL-*N*-METHYL BARBITURATE) AND UPON THE RESPONSE OF ALBINO MICE TO THE LATTER

The results of six experiments with pernoston are shown in Table I. With a daily dose of 125 γ of the hormone per normal female rat, it was necessary to continue the administration for about two weeks to get a significant difference in recovery time. However, with 500 γ, such a difference was found after one full week of treatment. Although the prolonged hormone medication shortened the female rat recovery time from pernoston, this time never approached the recovery time of the male rats, which is much shorter in the normal male rat. Whether still higher hormone doses could have shortened the female recovery time to the male level we cannot say, but we will return to this problem in Part II. It is also interesting that the percentage of delayed deaths in the hormone-treated females was only 6%, whereas in the normals it was 18%. However, even heavy doses of the hormone failed to completely prevent delayed deaths.

The first experiment in Table II shows that 10-day administration of only 62½ γ of testosterone acetate per rat daily definitely made such treated females recover faster, but did not influence the male rat response to evipal. Three experiments with much higher hormone dosage also failed to change the male response. In an experiment upon mice, the relatively large dose of 125 γ of male hormone per mouse daily for a week failed to influence the recovery time of female mice.

In Table III are shown the results of experiments upon two groups of adult female albino rats in case of which rats of Group 2 were given testosterone acetate before spaying and rats of Group 1 were given this hormone after spaying. With administration of 125 γ per rat daily for about one week, the

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‡ From the Department of Physiology and Pharmacology, College of Pharmacy, University of Nebraska, Lincoln, Nebr.

Table I.—Summary of the Results of the Intraperitoneal Administration of 50 Mg./Kg. of Pernoston (Sodium 2-Butyl-Bromallyl Barbiturate, 1% Solution) to 16 Groups Consisting of 12 Albino Rats Each, 3 to 5 Months Old, Which Had Been Given Varying Doses of Testosterone Acetate (Perandren)

The two values under each group in each test represent the average recovery time in hours and the actual number of delayed deaths. The testosterone acetate (perandren) was administered subcutaneously in sesame oil; the final dose was given 3 hrs. before the pernoston; thus, 5 doses indicates that the hormone was allowed to act slightly over 4 full days. The rats were observed over a period of 5 days for delayed deaths. One hormone-treated rat died acutely in Group 2. In case of the two female groups, the critical ratios were calculated in the usual manner from the average recovery times and their standard errors.

No. of Test	Normal Males		Normal Females		Hormone-Treated Females		Critical Ratio	Daily Gammas of Testosterone Acetate per Rat	No. of Doses
1	1.3	0	4.1	4	3.9	0	0.7	125	3
2	1.4	0	4.5	3	4.2	2	1.2	125	5
3	1.2	0	4.1	4	3.2	0	3.1	125	15
4	1.3	0	3.9	0	3.4	1	2.2	500	5
5	1.2	0	4.2	0	3.4	0	2.9	500	8
6	[a]	..	[a]	..	2.7	1	6.0	500	15
Total percentage of delayed deaths			18			6			

[a] The normal rats for Group 3 also served as controls for Group 6.

Table II.—Summary of the Results of the Administration of Sodium Evipal (Sodium Methyl-Cyclohexenyl-*N*-Methyl Barbiturate) to 10 Groups Consisting of 10 to 14 Rats Each and to 2 Groups Consisting of 14 and 15 Mice, Respectively, Which Had Been Given Varying Doses of Testosterone Acetate (Perandren)

The rats were from 3 to 6 months old, and the mice weighed from 20 to 28 Gm. each. In Groups 1 and 3, all were albinos; Group 4, albinos with a few hybrids; and Group 2, all hybrids. Values under each group are the average recovery time in hours. Sodium evipal concentrations were such that 0.5 cc. of solution was administered per 100 Gm. of body weight. Testosterone acetate (perandren) was administered as under Table I. One rat died acutely in the hormone-treated group under 4. The critical ratio between the two female groups under 1 was 3.1.

		Average Hours of Recovery					
No. of Test	Sodium Evipal, Mg./Kg.	*A*, Normal Males	*B*, Hormone-Treated Males	*C*, Normal Females	*D*, Hormone-Treated Females	Gammas of Testosterone Acetate per Rat or Mouse	No. of Doses
Rats							
1	80, ip.[a]	0.9	1.0	1.6	1.2	62.5	11, two daily
2	200, subc.[b]	2.8	2.6	...	...	125	8, one daily
3	200, subc.	2.4	2.4	...	...	125	11, one daily
4	200, subc.	2.7	2.8	...	...	500	11, one daily
Albino Mice							
5	180, subc.	...	...	1.8	1.8	125	8, one daily

[a] Intraperitoneal administration.
[b] Subcutaneous administration.

Table III.—Summary of the Results of Subcutaneous Administration of 100 Mg./Kg. of Sodium Evipal (Sodium Methyl-Cyclohexenyl-*N*-Methyl Barbiturate, 2% Solution) to Two Groups of 5½-Month-Old Albino Female Rats, 15 in Each Group at the Start

Group 2 was administered testosterone acetate (perandren) as indicated in the dose column before spaying, and Group 1 was treated after spaying. In Group 1, one rat died during the 5-week interval, and a second one during the last interval. In Group 2, one rat died actuely during the eighth test; it had lost much body weight since the previous test. The testosterone acetate (perandren) was administered and the critical ratios calculated as under Table I.

Group No.	Average Hours of Recovery										Gammas of Testosterone Acetate per Rat per Dose	No. of Doses
	1[a]	1	1	2	1½		1	2	5	2		
1	3.8	3.6	2.9	3.4	3.8	All Spayed	2.6[a]	3.4	3.0[b]	3.3	[a]125	9
											[b]125	8
2	3.6	3.5	2.4[c]	2.4[d]	3.7		3.4	3.2	3.6	3.5	[c]125	8
											[d]500	7
Critical Ratios:			3.7						4.3			

[a] Time interval in weeks between administrations of evipal.

Table IV.—Summary of the Results of Subcutaneous Administration of 100 Mg./Kg. of Sodium Evipal (Sodium Methyl-Cyclohexenyl-*N*-Methyl Barbiturate, 2% Solution) to Five Groups of Hybrid Rats

The hormone was administered subcutaneously in sesame oil (1 mg. of testosterone in 1 cc. of oil). In addition to the daily administration of testosterone propionate during the preceding 2 weeks as indicated, one dose was also given 3 hrs. before the evipal administration.

Group	No. of Rats	Age in Months	Sex	Average Hours of Recovery 17*	21	14	35	69	Daily Mg. of Testosterone per Rat (Given as the Propionate)
1	8	6–7	Male	1.7	1.9	1.8	1.8	...	... Not treated
2	7	6–7	Female	2.9	1.9[a]	2.3	2.7	...	... [a] 0.25 during preceding two weeks
3	8	11–12	Male	1.9	1.9	1.6[b]	1.5[c]	1.7	2.0 [b] 0.25 during preceding two weeks
									[c] Then 0.50 for two weeks
4	14	2	Male	1.0	1.1	1.3	1.3	1.6	1.7 The usual increase as untreated males become mature
5	14	2	Female	1.7	1.9	1.8	1.3[d]	2.5	2.9 [d] 0.25 during preceding two weeks

* Time interval in days between administrations of evipal.

hormone was very efficient in shortening the recovery time from evipal in both the normal and the spayed females. Five hundred gammas daily were given to the normal females for a week following the 125-γ administration; this raised the difference between the hormone-treated and untreated rats. In all cases the improvement induced by the male sex hormone had disappeared two weeks after stopping the hormone administration.

PART II. INFLUENCE OF VARYING DOSES AND DURATIONS OF ADMINISTRATION OF TESTOSTERONE PROPIONATE (PERANDREN) UPON THE RESPONSE OF HYBRID RATS TO SINGLE DOSES OF SODIUM EVIPAL AND OF NOSTAL (ISOPROPYL-BROMALLYL BARBITURATE)

While conducting experiments with testosterone acetate, the more potent propionate became available. We therefore substituted this drug to ascertain and compare its effects. The results of experiments with sodium evipal are presented in Table IV. A large dose of 250 γ of the propionate per rat daily for two weeks made the female rat response equal to that of the male. Also, for the first time, there was an indication of shortening the male response; apparently 500 γ of the propionate were not significantly more effective than 250 γ. These rats were about one year old.

Our earlier experiments (1) with urinary male hormone indicated that when the rat exhibited no sex difference to a barbiturate (barbital), the male hormone did not influence the recovery time. We therefore administered testosterone propionate to both male and female rats before giving nostal, in case of which the rat in previous tests had shown only slight sex difference. The results are shown in Table V. Although, for the first time in our experience, a clear-cut sex difference was apparent, both in the recovery time with nostal and the proportion of delayed mortality cases, the 10-day administration of 1 mg./Kg. daily of testosterone (given as the propionate) did not influence either the acute recovery time or the delayed mortality percentage. Control injections of sesame oil likewise had no effect upon these responses to nostal.

Table V.—Summary of the Results of Intraperitoneal Administration of 50 Mg./Kg. of Nostal (Isopropyl-Bromallyl Barbituric Acid, 1% Solution) Given as the Sodium Salt to Six Groups Consisting of 7 to 8 Hybrid Rats Each, 3 to 3½ Months Old

The testosterone propionate (perandren) was administered subcutaneously in sesame oil, and the final dose was given 3 hrs. before the nostal. The average for the females that were given sesame oil is omitted because one apparently normal rat took about 8 hrs. to recover; the average for the other six was 2.6 hrs. All three groups showed normal gains in body weights during the oil and hormone administration.

Group	Males: Average Hours of Recovery	Males: Delayed Mortality	Females: Average Hours of Recovery	Females: Delayed Mortality	Treatment
1	1.8	3/8	2.6	6/7	None
2	1.8	2/8	...	6/7	Sesame oil, 1 cc./Kg. daily, 11 doses
3	1.8	3/8	2.4	7/8	Testosterone propionate,[a] 1 mg./Kg. daily, 11 doses

[a] Calculated as testosterone.

PART III. EFFECTS OF CASTRATING AND OF VARYING DOSES AND DURATIONS OF ADMINISTRATION OF TESTOSTERONE PROPIONATE (PERANDREN) UPON THE ABILITY OF THE ALBINO RAT TO BUILD UP TOLERANCE TO AND TO DETOXIFY SODIUM PENTOBARBITAL

To elucidate further the increased resistance caused by male sex-hormone administration, we next employed a new technique developed by Holck and Fink (7). The rats are first given a small dose of sodium pentobarbital per Kg. of body weight, and every 90 min. the dose is increased by a small percentage. One can then compare the percentages of rats which develop tolerance and determine quite closely the highest dose each tolerant rat can detoxify in 90 min. By choosing the proper dosage, these authors and also Holck and Mathieson (8) found that nearly all normal adult males develop tolerance, that castration of such males diminishes the number which develop tolerance, that very few

normal adult females develop tolerance, except during pregnancy and the period of lactation, and, finally, that almost all one- or two-month-old rats of either sex develop tolerance, and that within these limits, the younger the rat, the more pentobarbital it can destroy in 90 min. The rats were allowed food and water throughout these tests.

Two male and two female rats, two months old, were given subcutaneous control injections of Ringer's solution; two other males and two other females were given one and one-half times this control dosage. At the end of 82 injections all of the rats were alive, but showed subcutaneous edema. One male receiving the larger saline dose died on the second day after the injections were stopped. Also, one one-month-old male rat was permitted to recover after receiving 80 doses of pentobarbital; it had apparently recovered an hour and fifteen minutes after the last dose and was growing well one week later.

In these earlier tests we started with 11.7 mg./Kg. and each dose increase was 3.22%. To avoid an excessive number of doses, we started with 10.9 mg./Kg. in the experiments in Parts III and IV, and employed 4.5% dosage increases; now only a very few of the young rats survived 60 doses, the maximum being 66 doses.

The results of our 14 experiments pertaining to Part III are presented in Table VI. The data of Group 3, Part *A*, Table VIII verify our previous finding that castration of adult male rats diminishes the percentage of rats which develop tolerance, but once tolerance has been established, the castrates detoxify pentobarbital as well as the normals. Similarly, we verified that spaying of adult female rats somewhat increased the percentage which develop tolerance (Group 10, Part *B*, Table VIII). However, spayed female rats were far below the normal males in their ability to develop tolerance.

Heavy dosage of testosterone propionate for three days did not influence the normal male rat response, but with the 10-day administration all male rats developed tolerance. Doubling the hormone dose was no more effective than the single dose of 1 mg./Kg. of body weight daily for ten days. In castrate males the 10-day medication again was somewhat more effective than the 3-day medication in distinctly increasing the percentage of rats that developed tolerance. The hormone did not increase the ability of tolerant castrated rats to de-

Table VI.—Summary of Experiments upon the Effects of Castration and of Varying Doses and Durations of Time of Administration of Testosterone Propionate (Perandren) upon the Ability of the White Rat to Build Up Tolerance to and to Detoxify Sodium Pentobarbital

The hormone was administered as a 1.19% or 2.39% solution, in sesame oil; 1 or 2 mg./Kg. of body weight, calculated as testosterone, was injected subcutaneously daily for the specified number of days prior to the pentobarbital administration, at least 90 min. before the pentobarbital medication, and thence daily until each rat had died. The barbiturate was given subcutaneously in 1% solution. Starting with 10.9 mg./Kg. of body weight, the dose was increased by 4.5% every 90 min., and thus continued until each rat had died. The animals averaged 7 months of age (range, 6 to 9 months), except the females which received 2 mg./Kg. of the hormone, which were 2 months older (8 to 10 months).

Groups	No. of Rats	Per Cent Developing Tolerance	Average Total Fatal Dose, Mg./Kg.	Tolerant Rats Only: No. of Rats	Average Total Fatal Dose, Mg./Kg.	Average Maximum Dose Detoxified in 90 Min., Mg./Kg.	Standard Error	Critical Ratio
Males								
1. Normal (*N*)	19	84	1081	16	1255	52	3.2	1.4 (1 & 2)
2. *N* + 3 day testosterone (T), 1 mg./Kg.	21	81	1173	17	1429	60	4.9	....
3. N + 10 day T, 1 mg./Kg.	22	100	1406	22	1406	..	...	....
4. N + 10 day T, 2 mg./Kg.	15	87	1228	13	1398	..	...	....
5. Castrates (C)	23	52	731	12	1284	..	...	....
6. C + 3 day T, 1 mg./Kg.	23	83	1063	19	1254	..	...	....
7. C + 10 day T, 1 mg./Kg.	22	95	1273	21	1329	..	...	....
Females								
8. Normal	23	9	206	2	895	..	...	....
9. N + 3 day T, 1 mg./Kg.	20	15	292	3	1192	..	...	....
10. N + 10 day T, 1 mg./Kg.	23	65	955	15	1386	..	...	....
11. N + 10 day T, 2 mg./Kg.	20	55	856	11	1436			
12. Spayed (S)	23	35	490	8	1202	52.2	2.1	3.0 (12 & 14)
13. S + 3 day T, 1 mg./Kg.	23	74	898	17	1162	48	2.1	4.1 (13 & 14)
14. S + 10 day T, 1 mg./Kg.	24	83	1353	20	1602	65	3.5	....

Table VII.—Summary of the Results from the Subcutaneous Administration 100 Mg./Kg. of Sodium Evipal (Sodium Methyl-Cyclohexenyl-*N*-Methyl Barbiturate, 2% Solution) to Two Groups Consisting of 11 Albino Male Rats Each, and to Two Groups Consisting of 14 Albino Female Rats Each, 3 to 4 Months Old

The hormone was administered as a 0.67% solution of estradiol dipropionate in a sesame oil; 1 mg./Kg. of body weight, calculated as estradiol, was injected subcutaneously daily for 10 days. The final dose was given 3 hrs. before the evipal.

Group	Average Weight, in Grams	Sodium Evipal, Average Hours of Recovery	Treatment (10 Days)	Weight	Hours of Recovery	Weight	Hours of Recovery	Weight	Hours of Recovery
Males		10[a]			24[a]		14[a]		
1	222	1.5	Sesame oil, 2 cc./Kg. daily	227	1.3	258	1.5	256	1.9
2	204	1.5	Estradiol, 1 mg./Kg. daily, 11 doses	172	2.2	192	2.7	201	3.0
Females									
3	159	2.5	Sesame oil, 2 cc./Kg. daily	159	2.6	179	2.8	...	...
4	159	2.5	Estradiol, 1 mg./Kg. daily, 11 doses	150	2.6	173[b]	2.6	...	...

[a] Time interval in days.
[b] One rat died two days previously; the average gain of the 13 rats was 24 Gm.

toxify pentobarbital. Concerning normal female rats, again the 10-day hormone treatment was much more effective than the one of only three days, and again 2 mg./Kg. were no better than 1. As to spayed female rats, the 10-day was somewhat more effective than the 3-day administration in markedly increasing the percentage of rats that developed tolerance. Whereas the average maximum dose detoxified in 90 min. was not influenced by the 3-day medication, it was significantly higher in the spayed females when the hormone was given for ten days.

PART IV. EFFECTS OF AGE, OF CASTRATION AND OF VARYING DOSES AND DURATIONS OF TIME OF ADMINISTRATION OF ESTRADIOL DIPROPIONATE (DI-OVOCYLIN) AND TESTOSTERONE PROPIONATE (PERANDREN) UPON THE ABILITY OF THE ALBINO RAT TO DETOXIFY EVIPAL AND TO BUILD UP TOLERANCE TO AND TO DETOXIFY PENTOBARBITAL

In the experiments upon the effect of 10-day administration of a large, daily dose of estradiol dipropionate (di-ovocylin) upon the response to sodium evipal, summarized in Table VII, there was a weight loss in both sexes, but the reduction was much greater in the males. Concerning the effect of this hormone upon the recovery time from sodium evipal, this was not altered in the females; but with the males it was markedly lengthened, even though the evipal dose was based upon the reduced body weight. Twenty-four days after discontinuing the hormone medication, all rats showed good gains in weight, but the males were not yet back to their original weights. Whereas now the recovery times from evipal were practically identical in the two female groups, with the treated males it was even longer than at the end of the hormone administration. Two weeks later the hormone-treated males had gained still further in weight, but their recovery time still was much longer than that of the controls. At the end of this last test with evipal all of the males were killed with chloroform.

Smear tests from epididimus material showed numerous sperms in all of the controls; this was true in only four of the hormone-treated rats; in four of the latter no sperms could be found and in three only very few. Inasmuch as a marked reduction in the size of testes was observed in six of the eleven hormone-treated males, we weighed all the testes. In the 11 controls, the average weight of testes was 0.81% of body weight; one of these rats had only one testis, and in two others the glands were of moderate size. In the hormone-treated rats the average was only 0.49% of body weight, and only four of the eleven rats had nearly normal percentages. However, there was no correlation between the size of the testes and the recovery time in these rats.

Again administering gradually increasing doses of sodium pentobarbital (see Part III), the effects of varying doses of estradiol dipropionate were compared in the two sexes. The first hormone dose was given ten days prior to the first sodium pentobarbital administration; the effect of this hormone dose was then followed up by taking vaginal smears; the second dose was given five days before beginning with pentobarbital, and smear tests again carried out twice; the third hormone dose was injected on the day prior to the pentobarbital medication and was followed by a vaginal smear test before the fourth hormone administration given three hours before the pentobarbital tests were commenced. Finally, the hormone was continued once daily in rats which developed tolerance to the barbiturate. All results are summarized in Table VIII, Parts *A* and *B*. All female rats gave positive vaginal smear tests (only one was doubtful) on the day the pentobarbital medication was started. The spayed females gave positive tests sooner with the 30 γ/Kg. of body

Table VIII.—Summary of Experiments upon the Effects of Age, of Castrating and of Varying Doses and Durations of Time of Administration of Estradiol Dipropionate (Di-ovocylin) and Testosterone Propionate (Perandren) upon the Ability of the Albino Rat to Build Up Tolerance to and to Detoxify Pentobarbital

The estrogenic hormone was administered as a 1.33% solution in sesame oil; 1 mg./Kg. of body weight, calculated as estradiol, was injected subcutaneously 10 days, 5 days, 1 day, and 3 hrs. prior to the pentobarbital administration, and thence daily until each rat had died. The testosterone propionate was administered in corresponding concentration in sesame oil and the dosage calculated as testosterone. It was administered daily beginning 10 days prior to, 3 hrs. before the pentobarbital administration, and thence daily until each rat had died. The barbiturate was given subcutaneously (1% solution). Starting with 10.9 mg./Kg. of body weight, the dose was increased by 4.5% every 90 min., and thus continued until each rat had died. The adult rats were from $3^1/_2$ to 8 months old, and the young rats were 7 to 10 weeks old. Comparing only the rats which developed tolerance the average maximum dose of sodium pentobarbital detoxified in 90 min. was 98 mg./Kg. for the 15 young normal female rats and only 74 mg./Kg. for the 11 young female rats treated with estradiol dipropionate.

PART A.—MALES

Groups	No. of Rats	Average Weight in Grams 10 Days Prior to Pentobarbital Administration	Day of Pentobarbital Administration	Per Cent Developing Tolerance	Tolerant Rats Only: Average Total Fatal Dose, Mg./Kg.	Tolerant Rats Only: No. of Rats	Tolerant Rats Only: Average Maximum Dose Detoxified in 90 Min., Mg./Kg.	Seminal Vesicles, All Rats Average, Mg./100 Gm.
1. Normal (*N*) adults	21	300[a]	308[a]	85	1087	18	51	306
2. *N* + estradiol (E), 30 γ/Kg., 4 doses	25	281	270	36	547	9	51	125
3. Castrate (C) adults	24	270	276	29	475	7	46	50
4. C + E, 30 γ/Kg., 4 doses	23	276	273	25	444	6	42	59
5. C + E, 30 γ/Kg., 4 doses + testosterone, 1 mg./Kg., 11 doses	24	278	284	83	1233	20	57	390
6. Normal young (Y)	12	154	176	100	2517	12	95	152
7. Y + E, 300 γ/Kg., 4 doses	14	155	155	85	1522	12	66	84

[a] Due to a misunderstanding only ten of the rats were weighed ten days before the pentobarbital experiment. The weights here given represent these ten rats. The average weights of all the 21 rats on the day of the pentobarbital administration was 294 Gm.

PART B.—FEMALES

Groups	No. of Rats	Average Weight in Grams 10 Days Prior to Pentobarbital Administration	Day of Pentobarbital Administration	Per Cent Developing Tolerance	Average Total Fatal Dose, Mg./Kg.	Uterus and Tubes, Average Mg./100 Gm. of Rat	Remarks on Vaginal Smear Tests before and on Pentobarbital Test Day
8. Normal (*N*) adults	26	197	192	0	113	330	Usual varied picture on and before test day
9. N + 30 γ/Kg. estradiol (*E*), 4 doses	26	188	188	4	149	283	Nine—four days after first dose of E, 15—day before, all + on test day
10. Spayed (S) adults	27	209	217	15	312	61	All negative
11. S + 5 γ/Kg. E, 4 doses	27	213	211	4	191	105	One—four days after first dose of E, one on day before and doubtful on test day
12. S + 30 γ/Kg. E., 4 doses	25	203	201	0	143	182	All + third day after first dose of E, all + on test day
13. Normal young (Y)	19	119	132	79	2020	164	Usual varied picture on and before test day
14. Y + 300 γ/Kg. E., 4 doses	19	122	130	58	1169	249	One—third day after first E dose, one—day before, all + on test day

weight than the normals. That our dosage was effective is also seen by the increased average weights of the uterus and uterine tubes in the spayed rats, varying with the dose, and that the large dose of 300 γ/Kg. increased these weights in the young normal females. The two junior pharmacy students who did our organ weighing had previously practiced the correct procedure upon four normal rats. In spite of this limited experience, we believe that the large average trends certainly can be considered significant. In the male rats the estradiol ester diminished the average size of the seminal vesicles markedly in both adult and young normal males, but had no influence upon these in castrated adult males.

As to body weight, estradiol caused a loss in the normal adult male rats, inhibited the growth of the young males, but had no certain effect upon any of

the female groups, or upon the male castrates. Concerning the effect upon resistance to pentobarbital, the estrogenic hormone markedly lowered the percentage of normal adult males which developed tolerance, clearly diminished the capacity of the young males to detoxify pentobarbital, decreased the percentage of spayed or of normal young female rats which developed tolerance, and, consequently, the average total fatal dose; it had no certain effect upon castrated males or upon normal adult females. Comparing only the young female rats which developed tolerance, the hormone decreased the average maximum dose of pentobarbital detoxified within 90 min. (see end of legend to Table VIII). When a dose of testosterone propionate (Group 5, Part *A*), previously found to be effective, was given together with a smaller dose of estradiol dipropionate to castrated adult male rats, the testosterone effect dominated in that the percentage which developed tolerance was markedly raised, the maximum dose detoxified within 90 min. by the tolerant rats was somewhat greater, and the seminal vesicles were increased markedly in size.

DISCUSSION

We have here established that testosterone acetate or propionate, when given daily in suitable doses for one or two weeks, causes significant shortening in the depression time in normal female rats and in castrated rats of either sex to the two barbiturates, pernoston and evipal; only with the propionate did we succeed in shortening the hypnosis by evipal in the normal male. Equally important is the observation that such prolonged hormone administration markedly increases the percentage of normal female and of castrated male or female rats which develop tolerance when pentobarbital is administered in very gradually increasing doses every 90 min. until each rat dies, and that in spayed females the hormone medication also increases the capacity to detoxify pentobarbital once tolerance is established; this latter needs confirmation.

The exact mechanism by which these changes in the rats are produced by the male sex hormone, and that by which effects of opposite nature are produced by castration in the two sexes must await future investigation. These involve the question of possible changed resistance of the nervous system to the barbiturates evipal, pernoston and pentobarbital. They necessitate further studies upon changes in the composition of the blood, especially in regard to calcium (6), directly or indirectly produced by the testosterone, because reports in the literature are conflicting concerning such changes. One may need to examine in more detail the ability of the liver to detoxify the mentioned barbiturates under the various conditions given. This plan is suggested by the reports of Deuel, *et al.* (9, 10, 11, 12), that the glycogen content of the liver of the normal males and spayed females was higher than in the normal adult females, that immature rats showed no sex difference in the glycogen content of the liver, and that testis hormone administration caused an increased glycogen content in this gland, whereas estrone had the opposite effect. In this connection we may note that estradiol, wherever any action could be detected by us, lowered the capacity to develop tolerance and to detoxify pentobarbital. Also that Holck, *et al.* (1), failed to accentuate the sex difference of the rat to evipal by a preliminary inanition period of 36 hrs., which causes faster depletion of liver glycogen in the female rat. A possible protection of the liver cells by testosterone is also in point (13).

In any case we have the additional problems of explaining: (*a*) why the male sex hormone failed to change the resistance of the rat to the related barbital (1) and nostal; (*b*) why castrated males, once they have developed tolerance, detoxify pentobarbital as well as normal male rats do; (*c*) why the synthetic male sex hormone failed to influence the resistance of female mice to evipal, toward which the resistance of female rats is easily increased. One should try different doses in mice before drawing a final conclusion, especially so because it has been reported recently that testosterone medication greatly increased their resistance to moderate doses of mercuric bichloride (13).

SUMMARY

1. Testosterone acetate in a dosage of 125 γ per rat daily for two weeks or 500 γ for one week significantly shortened the response of the rat to the barbiturate, pernoston. Eleven doses of 62.5 γ, given twice daily, shortened the response of normal female rats to evipal, but 10-day hormone treatment with twice this dose given daily, or 0.5 mg. daily for ten days, failed to influence the male rat response to this barbiturate. Likewise, 125 γ of the hormone daily per adult female mouse for a week failed to alter the response to evipal.

2. Treatment of about one week's duration with daily doses of 125 γ of testosterone acetate per rat significantly and to about the same extent shortened the response to evipal in both normal and spayed female rats.

3. Treatment for two weeks with 0.25 mg. per rat daily of the more powerful testosterone propionate shortened the response to evipal in adult and young female rats to that of the male level, and even reduced the depression time in the male; treatment of the male rats with double this daily hormone dose for two additional weeks was about as effective as the dose of 0.25 mg. per rat.

4. Ten-day treatment of male and female rats with 1 mg./Kg. of testosterone (given as the propionate) failed to influence the acute depression time and the incidence of delayed deaths following administration of the barbiturate, nostal, to which the untreated rats showed a measurable sex difference for the first time in our experience.

5. Three- or ten-day administration of 1 mg./Kg. of testosterone (given as the propionate) daily or double this dose for ten days did not influence the percentage of male rats which developed tolerance to gradually increasing doses of sodium pentobarbital. However, the lowered percentage of castrated male rats which developed tolerance was brought up to the normal level by either 3- or 10-day hormone administration in the dosage just given. The hormone administration did not increase the average maximum dose of pentobarbital that tolerant normal or castrated males detoxified within 90 min.

6. Whereas 3-day administration of 1 mg./Kg. of testosterone (given as the propionate) failed to increase definitely the percentage of normal female rats which developed tolerance to pentobarbital, 10-day treatment was signally effective in this respect; doubling the dose of the hormone did not cause further improvement. The somewhat increased percentage of tolerance consequent upon spaying of female rats was further increased by 3-day administration of the hormone and brought up to the male level by 10-day treatment. This latter also increased the average maximum dose of pentobarbital detoxified within 90 min. by the tolerant spayed females.

7. When estradiol (given as the dipropionate) was administered in a dosage of 1 mg./Kg. daily for ten days to normal male and female rats, it caused severe loss of body weight in the males and, later on, marked reduction in the size of the testes in most cases. Whereas this hormone did not influence the female reaction time to evipal, it markedly lengthened this in case of the males; this effect persisted at least six weeks after cessation of hormone medication when these male rats were again gaining in weight.

8. Five, thirty, and three hundred gammas, respectively, of estradiol per Kg. (given as the dipropionate) were administered ten, five and one day before, and on the day of beginning medication with gradually increasing doses of pentobarbital, and once daily thereafter to rats which developed tolerance. Vaginal smear tests were positive when the barbiturate was first given; also, definite effects were noted upon the average weights of the uterus and uterine tubes, and of the seminal vesicles. The only change in effects that could be detected was always a lessened ability to develop tolerance to pentobarbital, and, with the highest dose of hormone, a lowered average maximum dose of pentobarbital detoxified in 90 min. by tolerant young rats of either sex. When a heavy dosage of testosterone propionate was given simultaneously with a moderate dosage (30 γ/Kg.) of estradiol, which latter failed to influence the castrated male rat response, the beneficial action of the male hormone upon tolerance was observed in its usual degree.

We are indebted to Miss Lucille M. Mills and to Messrs. Lucien C. Kavan and James R. Weeks for aid in some of these experiments.

REFERENCES

(1) Holck, H. G. O., Kanan, M. A., Mills, L. M., and Smith, E. L., *J. Pharmacol.*, 60 (1937), 323.

(2) *Skand. Arch. Physiol.*, 77 (1937), 39.

(3) Holck, H., and Mathieson, D. R., *Am. J. Physiol.*, 133 (1941).

(4) Kinsey, V. E., JOUR. A. PH. A., 29 (1940), 387.

(5) Crabtree, D. G., Ward, J. C., and Welch, J. F., *Endocrinology*, 25 (1939), 629.

(6) Störtebecker, T. P., "Hormones and Resistance," Ejnar Munksgaard, Copenhagen, 1939, 294 pp. *Acta Pathologica et Microbiologica Scandinavica, Supplementum XLI.*

(7) Holck, H., and Fink, L. D., JOUR. A. PH. A., 29 (1940), 473.

(8) Mathieson, D. R., Thesis, University of Nebraska, 1941.

(9) Deuel, H. J., and Butts, J. S., *J. Biol. Chem.*, 100 (1933), 415.

(10) Deuel, H. J., and Gulick, M., *Ibid.*, 96 (1932), 25.

(11) Deuel, H. J., Samuels, L., and Gulick, M., *Proc. Exptl. Biol. Med.*, 30 (1932), 27.

(12) Deuel, H. J., *J. Biol. Chem.*, 99; *Scient. Proc. Soc. Biol. Chem.*, 27 (1932–1933), p. 35.

(13) Selye, H., *J. Pharmacol.*, 68 (1940), 454.

The Preparation and Vitamin A and Vitamin D Standardization of the Second U. S. P. Reference Cod Liver Oil by the U. S. P. Vitamin Advisory Board

The U. S. P. Reference Cod Liver Oil No. 2, a standard of reference for vitamin A and vitamin D, is now ready for distribution. This oil contains 1700 U. S. P. units of vitamin A and 115 U. S. P. units of vitamin D per gram. It has been prepared under the direction of the Vitamin Advisory Board and may be obtained from Dr. E. Fullerton Cook, Chairman of the U. S. P. Revision Committee, 43rd and Woodland Ave., Philadelphia, Pa.

Plans for providing this new reference cod liver oil were begun early in 1938 when it became apparent that the supply of U. S. P. reference cod liver oil was being depleted. At that time recognition was given to the principle that a standard for the assay of vitamins should preferably be the pure vitamin or a preparation made therefrom which could be readily reproduced and which might be suitable from the standpoint of stability and convenience. The International Standard for vitamin D now being distributed by the Health Organization of the League of Nations is of such a degree of purity. Some recent studies of vitamin A esters indicate that certain compounds of vitamin A may be suitable from the standpoint of stability and reproducibility as a standard for vitamin A. However, such pure preparations of vitamins A or D have not been available in sufficient quantities to supply the need for reference standards in this country and it was therefore decided to arrange for the preparation and standardization of another lot of reference cod liver oil. Owing to steadily increasing demands for samples of the reference cod liver oil for assay purposes, the stocks of the first lot of oil were depleted more rapidly than had been anticipated and it was necessary to issue samples of the new lot of oil just as soon as the standardization was completed.

In the early plans it was estimated that 200 gallons of destearinated oil would be a sufficient quantity of the new standard. With the equipment that was available for preparation of the oil it was found preferable to use a batch of 400 or 500 gallons for destearination and further processing. The fish liver oil secured was obtained from authoritative officials and is known to be exclusively the oil from the livers of *Gadus Morrhua*. This oil was obtained in lots of 120 gallons from the Norwegian Fisheries Research Station through the courtesy of Dr. Olav Notevarp, from the Fisheries Research Institute of Newfoundland through the courtesy of Dr. W. F. Hampton, and from Messrs. Crooks of London through the coöperation of Dr. J. C. Drummond. A fourth lot of 90 gallons was obtained from The Atlantic Coast Fisheries Company through the kindness of Dr. Harden F. Taylor. This specially selected oil was mixed, destearinated and packaged under carbon dioxide with relatively high pressure under the most ideal conditions obtainable and the oil has been stored under refrigeration continually from the day it was received in this country. The processing and packaging of the oil was done through the coöperation of E. R. Squibb and Sons.

In response to invitation to collaborate in the standardization of this oil more than 18 laboratories agreed to take part in the study. To these laboratories were sent samples of the oil to be standardized as well as International Standards for vitamins A and D to be used for reference in the biological assays. These assays were carried out according to the methods prescribed in the Second Supplement of the U. S. Pharmacopœia XI.

Complete reports from 18 laboratories have been received, 18 submitting results of vitamin D assays and 14 results of vitamin A assays, and interpretations of these records were used to set a value for the potency of the reference oil. A condensed summary of the reports received are presented in Tables I and II. In Table I are the vitamin A potencies assigned by each collaborator to the assay oil as well as the number of animals reported used by each of the laboratories responding. It will be noted that more than 1600 animals were used in the vitamin A assays alone. The arithmetical average of the values reported is 1787. The extreme values are 1000 and 2500. It is evident that the smaller numbers of animals were used by the laboratories reporting the highest potencies for the oil. The value 1700 was given the oil in consideration of the interpretation of the full reports submitted by the collaborators as well as in recognition of the preliminary reports of spectrophotometric collaborative studies which were yet to be completed.

Table I.—Condensed Summary of Vitamin A Collaborative Reports[a]

Laboratory No.	Vitamin A Value Assigned to Oil	Number of Animals Reported Used
1	1600	144
2	2000	86
3	1700	80
4	1475	112
5	2250	46
6	2000	158
7	1525	200
8	1800	56
9	1846	138
10	2500	40
11	2000	80
12	1000	72
13	1679	133
14	1650	320
	Average 1787.5	Total 1665

[a] The biological assay method used was that directed in the "Second U. S. P. XI Supplement" (1939), pages 132–138.

In Table II the vitamin D values assigned to the oil and the number of animals used by each laboratory are given. The average of these values is 114 and the extreme values are 100 and 130. The value of 115 units of vitamin D assigned to the reference cod liver oil seems well justified.

Table II.—Condensed Summary of Vitamin D Collaborative Reports[a]

Laboratory No.	Vitamin D Value Assigned to Oil	Number of Animals Reported Used
1	130	37
2	115	92
3	110	68
4	117	133
5	120	40
6	112	65
7	120	15
8	120	118
9	112	139
10	118	167
11	110	75
12	125	99
13	100	38
14	125	176
15	105	40
16	100	60
17	114	215
18	110	150
	Average 114.5	Total 1727

[a] The biological assay method used was that directed in the "Second U. S. P. XI Supplement" (1939), pages 132–138.

Fortunately it was possible to arrange through Dr. Chester D. Tolle, the Associate Referee on Vitamin D, an A. O. A. C. collaborative study of the new reference oil. This study, involving the comparison of the vitamin D potencies of the old and new reference oils by the A. O. A. C. chick method of assay, was conducted by 17 laboratories. The average of all vitamin D values assigned the new oil by the collaborators was 119 U. S. P. units per gram and of the 10 values ranging from 105 to 120 was 112 U. S. P. units per gram. These results were considered by the Vitamin Advisory Board since this reference standard is to be used in the chick assay of vitamin D carriers for poultry.

At the Second International Conference on Vitamins, held in London in June 1934 under the auspices of the Health Organization of the League of Nations, the advisability of preparing subsidiary standards for distribution within each country represented was discussed, with the view to reducing the amounts of the International Standards needed. In recognition of this view, in standardizing the U. S. P. Reference Oil No. 2 collaboration by laboratories outside of the United States was invited. The laboratory of the Pharmaceutical Society of Great Britain and the Laboratory of Hygiene, Department of Health, in Canada responded to this invitation. The reference cod liver oil now being distributed in Canada is identical with the U. S. P. Reference Cod Liver Oil No. 2 and the advantages of these two countries having a common standard are obvious.

The new reference oil was prepared in a manner identical with that used for the original oil. The original oil was destearinated, freed from moisture, placed in dry amber-colored bottles, subjected to vacuum and then charged with carbon dioxide, hermetically sealed and stored at a temperature close to zero degrees centigrade. This oil was widely used internationally as a standard both for bioassays and spectrophotometric determinations. There has been no evidence that this oil has deteriorated in any way since its original packaging. The Reference Oil No. 2 will be subjected to the same study in order to insure that there is no change in potency during its use.

Table III.—Vitamin A Value of U. S. P. Reference Cod Liver Oil No. 2 in Terms of Reference Oil No. 1 at 3000 U. S. P. Units per Gram

Laboratory No.	Determination on Raw Oil	U. S. P. Collaborative Saponification Procedure	Optional Saponification Procedure
1. Sa-40	1706	1772	1938
2. Sn-40	1446	1732	1574
3. Sl-40	1755	1841	1823
4. Sg-40	1831	....	1821
5. Si-40	1701	1393	....
6. Sh-40	1715	1640	1669
7. Sm-40	1739	1557	1607
8. Se-40	1962	1743	
9. Sf-40	1880	1968	1868
10. Sk-40	1766	1681	1919
11. Su-40	1620	1540	....
Average	1740	1687	1777

SUMMARY OF SPECTROPHOTOMETRIC STUDIES

The new Reference Cod Liver Oil was also subjected to a U. S. P. collaborative study involving the use of spectrophotometric methods for determining vitamin A. Eleven laboratories participated in this study. Determinations were made on the raw oil, on the non-saponifiable fraction of the oil prepared according to a suggested procedure, and on the non-saponifiable fraction prepared according to optional or preferred procedures. The results, in terms of

potency of the oil as calculated from the submitted data, are given in the accompanying table. The coefficient of variation in the results obtained by each of the procedures used has been determined. This value was found to be smallest for the determination on the raw oil, greater when the suggested saponification procedure was used and greatest for the optional saponification procedure results. However, the averages of the calculated potencies are in good agreement. The results of this study furnish further basis for the vitamin A value assigned the oil.

PHYSICAL FACTORS OF THE SECOND U. S. P. REFERENCE COD LIVER OIL

Dr. G. S. Jamieson, in charge of Oil, Fat and Wax Investigations, Agricultural Chemical Research Division of the Bureau of Agricultural Chemistry and Engineering, United States Department of Agriculture, has examined the new Reference Cod Liver Oil and submits the following report:

Refractive index at 25° C.	1.4771
Saponification value	188.6
Iodine number (Hanus)	172.1
Acid value	0.81
Unsaponifiable matter	0.60
Stearine (cold test)	None
Odor	Slight
Taste	Characteristic mild fishy flavor

A Phytochemical Study of the Root Bark and Fruit of *Cornus Nuttallii**

By Edward Krupski† and Louis Fischer‡

Cornus Nuttallii, commonly called Dogwood and a member of the Cornaceæ family, grows along the Pacific Coast from British Columbia to California, but thrives best in the Douglas Fir forests of the Puget Sound area. This tree was first observed by the botanist Nuttall and later named for him by Audubon (1). Torrey and Gray (2) published the first written description of this plant in their book "Flora of North America."

This tree usually grows from 20 to 30 feet high and from 6 to 8 inches in diameter. It has a dull ashy brown or reddish bark that forms thin scales on old trunks. The twigs are minutely hairy when young, later smooth, and dull red-purple in color. The leaves are deciduous, simple, opposite, three and one-half to five inches long and about one-half as wide. Button-like clusters of very small greenish-yellow flowers which ordinarily bloom in early spring are surrounded by four to six showy white bracts that are commonly taken to be the petals. The thin dry pulp of the drupes, which are bright red, and mature in clusters of 25 to 40 at the ends of the twigs, contains one or two seeds within a stony endocarp.

No record was found of *Cornus Nuttallii* ever having been used medicinally; however, *Cornus florida*, once official in the U. S. P., has been used for many years as a medicinal agent. The bark of the root and trunk of *Cornus florida* was used with some success as a substitute for Cinchona as an antiperiodic and tonic (3). Also, according to Ellis (4), the bark of *Cornus florida* was considered very valuable in the treatment of autumnal fevers; however, the active principle of neither species has been subjected to a pharmacological study in this respect.

EXPERIMENTAL

Collection of Material.—The fruits were gathered during the months of September and October in the immediate vicinity of Seattle. They were separated from the bracts and only the ripe, fully matured fruits were used in this investigation. After drying at a temperature of about 27° C., they were ground to the desired fineness.

The root bark was collected during September, in the vicinity of McCleary, Washington. After uprooting the trees, the roots were scraped clean and the bark peeled. The bark was well dried in the sun and then ground to a fine powder.

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† Submitted in partial fulfillment for a Master of Science Degree in Pharmacy.

‡ Associate Professor of Pharmaceutical Chemistry, College of Pharmacy, University of Washington, Seattle, Washington.

In the proximate analysis of the fruits and root bark, the procedures adopted were from the Methods of Analysis of the Association of Official Agricultural Chemists (A. O. A. C.), fifth edition, and the United States Pharmacopœia (U. S. P.), eleventh revision. All determinations were made in duplicate, the results averaged and reported in percentage of air-dried material.

Moisture.—Two methods were used to determine the moisture; in the first, the ground material was dried at 135° C. for two hours in an electric oven; the other procedure was the official toluene method as described in the U. S. P. The following results, expressed in percentage, were obtained by the two procedures.

	Bark	Fruit
Electric oven method	12.36	6.70
Toluene method	9.88	5.49

Selective Extraction.—Samples of the dried bark and fruit were extracted in a Soxhlet apparatus by the selective extraction method. The percolates were evaporated spontaneously and dried to constant weight in a desiccator. The volatile and non-volatile extracts were also determined and the results expressed, in per cent, as total, non-volatile, and volatile extract.

Selective Extraction of Bark

	Total Extract	Non-Volatile Extract	Volatile Extract
Petroleum ether	1.16	1.02	0.14
Ether	5.84	5.62	0.22
Chloroform	3.78	3.53	0.25
Ethyl acetate	9.02	8.11	0.91
Alcohol	10.14	8.56	1.58
Water	10.93	..	..

Selective Extraction of Fruit

	Total Extract	Non-Volatile Extract	Volatile Extract
Petroleum ether	21.80	21.66	0.14
Ether	2.59	1.80	0.79
Chloroform	0.61	0.48	0.13
Ethyl acetate	8.29	7.47	0.82
Alcohol	3.77	3.33	0.44
Water	8.04	..	..

Ash.—The total ash, water insoluble ash and acid insoluble ash were determined on the ground bark and fruit by methods described in the A. O. A. C. The results obtained, expressed as per cent, were as follows:

	Bark	Fruit
Total ash	9.15	3.23
Water insoluble ash	5.75	2.01
Acid insoluble ash	0.43	0.43

Reducing Sugars.—The quantitative method for determining reducing sugars listed under plant analysis in the A. O. A. C. was used. The weighed samples were neutralized with calcium carbonate and extracted with 80 per cent alcohol. After evaporating the alcohol, the residue was taken up with water and decolorized with lead acetate solution. The excess lead was removed and the reducing sugars were determined in a 50-cc. aliquot using the Munson-Walker method. Calculated in per cent of invert sugar the results were: bark, 6.56; and fruit, 3.75.

Sucrose.—The sucrose of the fruit was determined using the hydrochloric acid inversion method of the A. O. A. C., while the sucrose of the bark was obtained by the official method employing the use of the enzyme invertase.

Results of the sucrose determinations, in per cent, were: bark, 1.50; and fruit, 2.97.

Starch.—The A. O. A. C. quantitative method for the determination of starch was used. The residue remaining after removal of the sugars by hot alcohol was placed in water, warmed to gelatinize the starch and then hydrolyzed with Taka-diastase. After repeated treatment with Taka-diastase, the solution was filtered and further hydrolyzed with hydrochloric acid. The dextrose, present in an aliquot of the hydrolyzed solution, was determined by the Munson-Walker method. The amount of starch present was calculated and found to be 3.97 per cent in the bark and 3.97 per cent in the fruit.

Pentosans.—Using the A. O. A. C. procedure, samples of the bark and fruit were treated, as directed, with 12 per cent hydrochloric acid. Upon complete distillation, the distillate was treated with phloroglucin and from the weight of furfural-phloroglucide the amount of pentosans was calculated. The results, in per cent, were: bark, 10.56; and fruit, 16.55.

Crude Fiber.—This determination was made using the A. O. A. C. procedure which consisted of removing the ether-soluble constituents from weighed portions, then subsequently treating the residue with 1.25 per cent sulfuric acid, followed by digesting with the same strength sodium hydroxide. The loss in weight upon ignition of the acid and alkali treated residue was calculated as crude fiber. The results, in per cent, were as follows: bark, 14.24; and fruit, 26.39.

Protein.—The nitrogenous matter was determined upon the bark and fruit by the Kjeldahl-Gunning-Arnold method. The results were expressed as per cent of nitrogen and per cent of protein.

	Bark	Fruit
Nitrogen	1.66	1.03
Protein	10.36	6.44

Fixed Oil.—A fixed oil was obtained from the fruit by extracting with petroleum ether, using a Soxhlet apparatus. The oil was purified by refluxing an alcoholic solution with purified animal charcoal. After filtering, the solution was allowed to evaporate spontaneously until the odor of alcohol could no longer be detected. The following constants were determined using procedures as described in the A. O. A. C.:

Saponification number	191.45
Acid value	7.09
Ester number	184.36
Iodine number (Hanus method)	90.48
Specific gravity, 20° C.	0.9150
Index of refraction, 20° C.	1.5698

Cornin.—The glucoside, cornin, was extracted using the following procedure. The bark was repeatedly extracted with hot water until the aqueous extract was nearly colorless. The combined extracts were treated with lead acetate, evaporated to a pilular consistency under reduced pressure and extracted with acetone. The acetone was removed by distillation and spontaneous evaporation until crystals of cornin were obtained. These crystals, when recrystallized from acetone, melted at 179° C. The crystals were acetylized using the method of Reichert and Hoffman (5). One part of cornin, 10 parts of cold pyridine and 2 parts of acetic anhydride were mixed and allowed to stand in the cold for 18 hours. Then, while cooling, 75 parts of a 5 per cent solution of sulfuric acid were added. The acetylized product was washed with water, recrystallized from alcohol, and its melting point found to be 134° C.

Further evidence that this product was cornin are the facts that it was found to have an optical rotation of −180.4°, and that it reduced Fehling's solution and ammoniacal silver nitrate solution. Miller (3) reported comparable results for cornin isolated from the root bark of *Cornus florida*. The bark of *Cornus Nuttallii* was found to contain about one per cent of cornin.

Scyllitol.—Scyllitol, a polyhydroxy alcohol, was isolated from the bark by the following procedure. The drug was first macerated with water, then a sufficient quantity of sodium carbonate solution was added to neutralize any free acid present. The bark was then percolated with water until completely exhausted. The percolate was treated with a solution of lead acetate, evaporated under reduced pressure, and extracted with 95 per cent alcohol. The alcoholic solution was partly concentrated and then set aside to allow the scyllitol to crystallize. After several recrystallizations from alcohol, a product, amounting to 0.41 per cent, was obtained that melted at 319° C. Scyllitol was further identified by its acetyl derivative, melting point 291° C. The melting point of scyllitol and the acetyl derivative agree in both cases with those reported by Hann and Sando (6) from *Cornus florida*.

Alkaloids.—In testing for the presence of alkaloids the bark and fruit were extracted separately with one per cent tartaric acid solution. The extracts of each were concentrated, made ammoniacal and extracted with a mixture of one part of chloroform and three parts of ether. After shaking out the organic solvent mixture with one per cent sulfuric acid, the acidic solution was tested with various alkaloidal reagents and in each case negative results were obtained.

SUMMARY

The root bark and fruit of *Cornus Nuttallii* were submitted to proximate analysis. Per cents of moisture, ash, sugars, starch, pentosans, crude fiber and protein are reported.

In the study of the root bark, about one per cent of a glucoside was isolated, purified and identified as cornin by some of its physical properties and the melting point of the acetyl derivative.

The root bark yielded 0.41 per cent of the polyhydroxy alcohol, scyllitol, which was confirmed by the melting point of the alcohol and its acetyl derivative. Neither the fruit nor the bark gave any indication of positive results when examined for the presence of alkaloids.

A fixed oil was separated from the fruit and several of the constants were determined.

REFERENCES

(1) Audubon, J. J., "The Birds of America," The Macmillan Company, New York, N. Y., 1937, p. 367.

(2) Torrey, J., and Gray, A., "Flora of North America," Vol. I, John Wiley & Sons, Inc., New York, N. Y., 1838–40, p. 652.

(3) Miller, E. R., JOUR. A. PH. A., 17 (1928), 744–750.

(4) Ellis, C., *Am. J. Pharm.*, 7 (1835), 265.

(5) Reichert, B., and Hoffman, W., *Arch. Pharm.*, 275 (1937), 474.

(6) Hann, R. M., and Sando, C. E., *J. Biol. Chem.*, 66 (1926), 399–402.